Optimization of monitoring and management of anticoagulant therapy

JOSEPH S. BIEDERMANN

Optimization of monitoring and management of anticoagulant therapy

Joseph Siegmund Biedermann

Erasmus MC
University Medical Center Rotterdam

ISBN: 978-94-6295-769-5
Lay out: Wendy Schoneveld || www.wenziD.nl
Printed by: Proefschriftmaken || Proefschriftmaken.nl

Printing of this thesis was financially supported by: The Federation of Dutch Thrombosis Services (FNT)

Optimization of monitoring and management of anticoagulant therapy

Optimalisatie van monitoring en management van antistollingstherapie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
21 november 2017 om 11:30 uur

door

Joseph Siegmund Biedermann

Geboren te Amstelveen

Erasmus University Rotterdam

PROMOTIECOMMISSIE:

Promotor:	Prof.dr. F.W.G. Leebeek
Overige leden:	Prof.dr. H. ten Cate
	Prof.dr. S.C. Cannegieter
	Prof.dr. K. Meijer
Copromotor:	Dr. M.J.H.A. Kruip

TABLE OF CONTENTS

CHAPTER 1

General introduction and outline of the thesis

THE HEMOSTATIC SYSTEM

The hemostatic system is a complex balanced system that triggers clot formation to prevent blood loss after trauma. Adequate clot formation requires both the generation of a primary platelet plug (primary hemostasis) and fibrin-mediated clot stabilization and strengthening (secondary hemostasis). Finally, after wound healing, the thrombus is resolved to ensure continuous blood flow (fibrinolysis). Disturbance of the hemostatic balance can result in either hypocoagulability, associated with an increased bleeding risk, or hypercoagulability, associated with an increased risk of thrombosis.

THROMBOSIS

Thrombosis is the presence of a blood clot in an artery (arterial thrombosis) or vein (venous thrombosis) compromising distal blood flow. Importantly, thrombosis is the major underlying mechanism of cardiovascular diseases such as myocardial infarction, ischemic stroke and venous thromboembolism, with a major impact on worldwide morbidity and mortality (1). Three main components, together commonly known as Virchow's triad, have been identified contributing to the pathogenesis of thrombosis: alterations in the constituents of the blood (hypercoagulability), diminished blood flow (stasis), and endothelial damage (vessel wall) (2). Historically, arterial and venous thrombosis were regarded as different diseases from a pathophysiological perspective, arterial thrombosis mediated primarily by platelet aggregation (primary hemostasis) and venous thrombosis mainly mediated by fibrin formation (secondary hemostasis). More recent studies have shown that arterial and venous thrombosis also share common risk factors such as increasing age, obesity, smoking, inflammatory markers and increased clotting factor levels (3-7).

The treatment of arterial and venous thrombosis differs depending on the relative importance of different components of Virchow's triad to its pathophysiology. For hypercoagulability due to altered blood composition or stasis, such as in patients with atrial fibrillation, venous thrombosis and mechanical heart valves, anticoagulant treatment is indicated, whereas for myocardial infarction, triggered by rupture of an atherosclerotic plaque and consequent platelet activation, antiplatelet therapy is the most commonly used treatment. This thesis will now further focus on anticoagulant treatment and monitoring and management of anticoagulant therapy.

ANTICOAGULANT THERAPY

Anticoagulants exert their effect by downregulating thrombin-mediated fibrin formation, the endpoint of secondary hemostasis. Several anticoagulants are currently available, with different mechanisms of action. Unfractionated heparin, low-molecular-weight heparin (LMWH) and the pentasaccharide fondaparinux bind to and activate

the natural anticoagulant antithrombin, thereby causing a conformational change and increasing its inhibition of both thrombin and factor Xa (unfractionated heparin) or primarily factor Xa (LMWH, fondaparinux) (8). The direct oral anticoagulants (DOACS) directly inhibit thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, edoxaban) and have been registered for different indications in the last decade (9). Despite the increasing use of DOACS, vitamin K antagonists (VKA) are still the most commonly used anticoagulant worldwide. Vitamin K antagonists are inhibitors of the enzyme vitamin K epoxide reductase (VKOR) (10-12). This enzyme catalyzes the reduction of oxidized vitamin K to vitamin K hydroquinone, a necessary cofactor for vitamin K-dependent **γ**-carboxylation of clotting factors II (FII), VII (FVII), IX (FIX) and X (FX) and the natural anticoagulants protein C and protein S (10-13). Inhibition of VKOR by VKA results in a deficiency in these factors by inhibiting the recycling of oxidized vitamin K (10-12). Different VKA agents are widely available and used (warfarin, acenocoumarol and phenprocoumon), of which only acenocoumarol and phenprocoumon are registered and used in the Netherlands. The most important difference between these agents are their half-lives, ranging from 2-8 hours for the short acting acenocoumarol to up to 156-178 hours for the long-acting phenprocoumon (13). Due to many food and drug interactions, the variable dose-response between patients and within patients, and the relative narrow therapeutic window of these drugs, monitoring the anticoagulant effect of VKAs is pivotal for safe and effective treatment.

ASSESSMENT OF THE ANTICOAGULANT EFFECT OF VKA

Historically, the Quick and later the Owren's prothrombin time (PT), as determined by mechanical or photo-optical clot detection, have been used to monitor the anticoagulant effect of VKA. The Quick prothrombin time evaluates the extrinsic and final common pathway of the coagulation cascade and is sensitive for deficiencies in fibrinogen, FII, FV, FVII and FX, while the Owren's PT is only sensitive for deficiencies in FII, FVII, and FX due to the addition of adsorbed bovine plasma to its reagent (14). To measure the PT, calcium chloride and a thromboplastin reagent, containing tissue factor and phospholipids, are added to citrated platelet poor plasma in order to induce fibrin formation through activation of the extrinsic pathway. Thromboplastins can be isolated from tissue homogenates from animal or human source (tissue-extract thromboplastins) or prepared by reconstituting recombinant tissue factor in a synthetic phospholipid medium (recombinant thromboplastins). Due to lack of standardization in the preparation of tissue-extract thromboplastin reagents in the past, PT results between laboratories showed considerable discrepancies using identical plasma samples, which may partly be explained by differences in thromboplastin concentration and contamination during the preparation (15). As a result, the required VKA dose, based

on the PT result, also differed between thromboplastins reagents. An early study showed substantial geographical differences in mean daily warfarin dosages between patient groups, which turned out to be due to differences in used thromboplastin reagents (16). To overcome differences between laboratories in PT results due to the use of different thromboplastins, a derivative of the PT, the INR, which is short for the 'International Normalized Ratio', was adopted in the late 1970's and is still considered the gold standard for monitoring treatment with VKA (15). The INR is a ratio of the patient's prothrombin time (PT) to the mean normal prothrombin time (MNPT) as determined in at least 20 healthy volunteers, corrected for the international sensitivity index of the used thromboplastin reagent;

$$INR = \left(\frac{PTpatient}{MNPT}\right)^{ISI} \quad (15).$$

The sensitivity index of the used thromboplastin is determined by calibration against the reference thromboplastin from the World Health Organization using the manual tilt-tube method (15). Since the 1990's, several point-of-care (POC) devices have become available, measuring the INR directly in capillary whole blood using different test principles such as impedance measurement or electrochemical clot detection (17). Although different POC devices are currently available for POC INR measurement, the CoaguChek XS (Roche Diagnostics, Mannheim, Germany) is the most frequently used and studied POC device for INR measurement (17).

MONITORING OF VKA THERAPY

During VKA therapy, the INR is regularly measured and the VKA dose adjusted based on the INR result to keep the INR of a patient within a therapeutic INR range. The therapeutic INR range depends on the treatment indication and the individual bleeding and thrombotic risk of the patient. For patients with atrial fibrillation or venous thrombosis, the therapeutic INR range is usually set at 2.0-3.0, while for patients with mechanical heart valves, the therapeutic INR range is normally set at 2.5-3.5 (18). Until recently, the upper limits of the therapeutic INR ranges in the Netherlands were set slightly higher (2.0-3.5 and 2.5-4.0, respectively) (19), but these have been adjusted to the international standards since January 2016 (20). Regular monitoring of the INR and adjustment of VKA therapy when the INR is out of range is necessary, since the risk of thrombotic complications sharply increases at subtherapeutic INR levels (INR <2.0), while the risk of bleeding complications significantly increases at supratherapeutic INR levels (INR >3.5) (21,22). The importance of monitoring and adjustment of VKA dose to keep patients within their therapeutic range is illustrated by the fact that INR stability and the quality of anticoagulation control during VKA therapy is strongly associated

with the risk of both thrombotic and bleeding complications (23). Quality of anticoagulation control can be assessed using different methods, of which the most frequently used is the percentage of time in therapeutic range (TTR in %), as determined using the Rosendaal linear interpolation method (24). A TTR of at least 70% is considered as good quality of anticoagulation control (23,25).
In the Netherlands over 450,000 patients are currently receiving treatment with VKA (26). These patients are either monitored by a unique organization of anticoagulation clinics or measure their INR themselves using POC devices. In case of INR self-monitoring, slightly more than half of these patients (56%) communicate their INR to an anticoagulation clinic, which adjusts the VKA dose for them when the INR is outside the therapeutic range (self-monitoring), while the other patients (44%) also adjust the VKA dose themselves (self-management) (26). Although INR self-monitoring and self-management are both associated with better anticoagulation control and clinical outcome, relatively few patients perform INR self-monitoring or INR self-management (approximately 15-20% in the Netherlands), due to many reasons such as comorbidity, inability to measure the INR, or cognitive impairment (26,27).

ALTERNATIVES TO ANTICOAGULANT TREATMENT

The downside of anticoagulant therapy, irrespective of the chosen agent, is an increased risk of major bleeding during exposure. Major bleeding occurs in 2-5 per 100 patients per year during anticoagulant therapy of which approximately 5-10% are fatal. (28-30). Although the risk of thrombosis outweighs the bleeding risk in patients with mechanical heart valves and patients with non-valvular atrial fibrillation, justifying lifelong treatment, determining the optimal treatment duration after a first venous thrombotic event remains challenging (18). Patients with a first venous thrombotic event used to receive 3-6 months of therapy with anticoagulants after which anticoagulant treatment was discontinued. However, VTE recurrence rates after discontinuing anticoagulant therapy are high, and recurrent VTE is associated with significant morbidity and mortality (3,31). Although prediction models for recurrent VTE have been developed, and the nature of the index event (provoked or unprovoked) and male sex have been identified as important risk factors for recurrence, individualized treatment based on reliable prediction is still difficult to achieve (3). Nowadays, patients with unprovoked VTE without a high bleeding risk receive anticoagulant treatment for an indefinite time due to their high risk of recurrence, but safer anticoagulant drugs that lower the risk of recurrent VTE and have fewer bleeding complications are urgently needed. Safer anticoagulant drugs should substantially reduce the number of major and fatal bleedings in these patients (28-30).

As mentioned earlier, increasing evidence suggests pathophysiological overlap between arterial and venous thrombosis. Apart from shared risk factors, patients with a recent arterial thrombotic event are also exposed to an increased risk of venous thrombosis and vice versa (32-34). More importantly, the use of HMG-CoA reductase inhibitors, or statins, known for their protective effects on primary and recurrent arterial thrombosis, has also been associated with a decreased VTE risk (35). In the Jupiter trial, healthy individuals with high C-reactive protein serum levels but normal cholesterol levels were randomized between 20mg rosuvastatin once daily or placebo (36). Remarkably, in this trial rosuvastatin was not only associated with a significant reduction of arterial thrombosis, but also significantly reduced the risk of a first symptomatic VTE by approximately 40% (36). Since then several observational studies have linked statin use to a reduced risk of both first and recurrent VTE (37). However, the mechanism behind this risk reduction is not clear since high cholesterol levels are not associated with an increased VTE risk (5,38). Furthermore, a meta-analysis of randomized clinical trials, comparing statin use to no use and with other end points than VTE, showed no significant association between statin use and reduced VTE risk (39). Several antithrombotic effects of statins have since then been shown in animal models and in-vitro studies, including enhanced fibrinolysis and antiplatelet effects (40). However, most of these studies were small, non-randomized studies, none of which performed in VTE patients, or specifically investigating risk factors for recurrent VTE, such as high FVIII levels. Therefore, more research is needed before statin therapy can be considered as candidate for VTE prevention.

AIM AND OUTLINE OF THE THESIS

The aim of this thesis is to investigate several issues related to monitoring and management of anticoagulant therapy. More insight into these factors, amongst others, the safety of POC INR monitoring in unselected patients, thromboplastin-mediated INR stability and different calculation methods for quality assessment of VKA treatment, may improve both the quality of anticoagulation control as well as experienced treatment quality in these patients and may therefore improve long-term clinical outcome. This thesis consists of three parts.

The first and second part of this thesis focus on the monitoring and management of therapy with vitamin K antagonists. Point-of-care (POC) devices can rapidly provide INR results and are less invasive compared to traditional laboratory INR measurement for which venipuncture is necessary to obtain a plasma sample. Despite advantages for both patients and anticoagulation clinics, POC devices have almost exclusively been used by patients performing INR self-monitoring, which is only a minority (approximately 15-20%) of patients treated with VKA in the Netherlands (26). Quality of anticoagulation

control should be adequate during POC INR monitoring and clinical outcome at least comparable to laboratory INR monitoring, both in self-monitoring patients as well as in patients monitored with POC devices by anticoagulation clinics. First, we assess the analytical and clinical agreement between INR results derived from a point-of-care device (CoaguChek XS using capillary blood) and a frequently used laboratory method in the Netherlands (Hepato Quick, using citrated plasma) in a large cohort of VKA users (Chapter 2). Agreement between measurement methods is pivotal, since significant INR discrepancies between methods can directly affect dosing decisions and therefore INR stability during VKA treatment. Therefore, we investigate the variability, defined as the coefficient of variation (CV in %) of the INR, in both self-monitoring and non-self-monitoring patients during CoaguChek XS monitoring, who receive a constant dose of VKA (Chapter 3). The average biological within-subject variation (CVb in %) of the INR can be used to derive imprecision goals for POC devices and to determine if this monitoring method is precise enough for patient monitoring in daily practice. The precision of a measurement method (CV%) should not exceed 50% of the biological within-subject variation of the analyte. Subsequently, we investigate the impact of introduction of point-of-care INR monitoring on quality of treatment in non-self-monitoring patients (Chapter 4). In this study, both the quality of anticoagulation control as well as clinical outcome during point-of-care will be compared to outcomes during standard laboratory INR monitoring. Hereby we will assess both the safety and efficacy of POC monitoring by anticoagulation clinics compared to current standards. Then, we explore the sensitivity of different commercially used thromboplastins reagents to clotting factor VII (Chapter 5). This is studied since differences between thromboplastin reagents regarding sensitivity to FVII have been suggested as explanation for INR discrepancies between different measurement methods. Furthermore, INR stability during VKA treatment has also shown to be thromboplastin reagent dependent. Our hypothesis is that recombinant human thromboplastins are more sensitive to FVII than tissue-derived thromboplastins, which could affect stability of VKA treatment depending on the thromboplastin used for patient monitoring. Regarding management of patients during VKA treatment, we analyze the bleeding risk associated with the use of different low-molecular-weight heparin (LMWH) agents during combined treatment with VKA and LMWH, in a large cohort of patients with acute venous thrombosis (Chapter 6). Due to the high number of patients treated with LMWH worldwide, recognizing differences in bleeding risk associated with the use of different LMWH agents may reduce the number of bleedings in the future. In chapter 7 we compare two different methods (linear interpolation method and the cross-sectional proportion of INR results within the therapeutic range) for assessment of therapeutic quality control in patients on VKA using data from the Federation of Dutch Thrombosis Services. Monitoring the

quality of anticoagulation control in their patients is essential for clinics. Both methods have been used to report treatment quality but their level of agreement has not been extensively studied. Finally, we will try to identify predictors of bleeding and evaluate clinical outcome after dental procedures in patients on VKA (Chapter 8), because dental procedures are among the most common invasive procedures performed in these patients. Therefore, identification of risk factors and improving perioperative management may improve outcome in daily practice. Furthermore, insight in risk factors for adverse outcomes may also be extrapolated to other surgical procedures. The third part of this thesis focuses on the potential use of statins for secondary prevention of venous thromboembolism (VTE) after discontinuation of anticoagulant therapy. The recurrence rate after stopping anticoagulant therapy after a first VTE is approximately 30% within 5 years (3) and is, amongst others, influenced by the nature of the index event (provoke/unprovoked), gender, and site of origin. Although statin use has been linked to a reduced risk of recurrent VTE in several observational studies, the mechanism behind this risk reduction is currently unclear and the quality of the currently available evidence low, based on the observational nature of these studies (36). Furthermore, the lack of a clear pathophysiological mechanism through which statins may decrease VTE risk has been an important reason for a lack of clinical trials in this area. First, a general introduction will be given describing current evidence for statin use for VTE prevention from a pathophysiological and epidemiological point of view (Chapter 9). Second, we investigate the impact of initiation of statin therapy during VKA treatment on VKA maintenance dose (Chapter 10). Alterations in VKA maintenance dose after initiation of statin therapy could suggest anticoagulant properties of statins or a significant drug-drug interaction. By comparing the effect of different statins on VKA maintenance dose, both immediately and after several months, more insight can be obtained whether statins exert anticoagulant effects. Finally, we study the effect of statin therapy on platelet reactivity (Chapter 11) and several different coagulation factors associated with recurrent VTE, in patients with a history of VTE (Chapter 12), in order to unravel potential mechanism through which statins may reduce VTE risk. Finally, we will discuss the findings of this thesis, put them in a clinical perspective and reflect on the implications of these findings for future anticoagulant treatment and research (Chapter 13).

REFERENCES

1. ISTH Steering Committee for World Thrombosis Day. Thrombosis: a major contributor to the global disease burden. J Thromb Haemost. 2014;12(10):1580-90.
2. Bagot CN, Arya R. Virchow and his triad: a question of attribution. Br J Haematol. 2008;143(2):180-90.
3. Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica. 2007;92(2):199-205.
4. Cosmi B, Legnani C, Cini M, Favaretto E, Palareti G. D-dimer and factor VIII are independent risk factors for recurrence after anticoagulation withdrawal for a first idiopathic deep vein thrombosis. Thromb Res. 2008;122(5):610-7.
5. Holst AG, Jensen G, Prescott E. Risk factors for venous thromboembolism: results from the Copenhagen City Heart Study. Circulation. 2010;121(17):1896-903.
6. Quist-Paulsen P, Naess IA, Cannegieter SC, Romundstad PR, Christiansen SC, Rosendaal FR, et al. Arterial cardiovascular risk factors and venous thrombosis: results from a population-based, prospective study (the HUNT 2). Haematologica. 2010;95(1):119-25.
7. Timp JF, Lijfering WM, Flinterman LE, van Hylckama Vlieg A, le Cessie S, Rosendaal FR, et al. Predictive value of factor VIII levels for recurrent venous thrombosis: results from the MEGA follow-up study. J Thromb Haemost. 2015;13(10):1823-32.
8. Masuko S, Linhardt RJ. Chemoenzymatic synthesis of the next generation of ultralow MW heparin therapeutics. Future Med Chem. 2012;4(3):289-96.
9. Morotti A, Goldstein JN. New Oral Anticoagulants and Their Reversal Agents. Curr Treat Options Neurol. 2016;18(11):47.
10. Jin DY, Tie JK, Stafford DW. The conversion of vitamin K epoxide to vitamin K quinone and vitamin K quinone to vitamin K hydroquinone uses the same active site cysteines. Biochemistry. 2007;46(24):7279-83.
11. Tie JK, Stafford DW. Structure and function of vitamin K epoxide reductase. Vitam Horm. 2008;78:103-30.
12. Tie JK, Stafford DW. Structural and functional insights into enzymes of the vitamin K cycle. J Thromb Haemost. 2016;14(2):236-47.
13. Beinema M, Brouwers JR, Schalekamp T, Wilffert B. Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon. Thromb Haemost. 2008;100(6):1052-7.
14. Horsti J. Has the Quick or the Owren prothrombin time method the advantage in harmonization for the International Normalized Ratio system? Blood Coagul Fibrinolysis. 2002;13(7):641-6.
15. Poller L. International Normalized Ratios (INR): the first 20 years. J Thromb Haemost. 2004;2(6):849-60.
16. Poller L, Taberner DA. Dosage and control of oral anticoagulants: an international collaborative survey. Br J Haematol. 1982;51(3):479-85.
17. Christensen TD, Larsen TB. Precision and accuracy of point-of-care testing coagulometers used for self-testing and self-management of oral anticoagulation therapy. J Thromb Haemost. 2012;10(2):251-60.
18. Holbrook A, Schulman S, Witt DM, Vandvik PO, Fish J, Kovacs MJ, et al. Evidence-based management of anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e152S-84S.
19. Kwaliteitsinstituut voor de Gezondheidszorg CBO. Diagnostiek, preventie en behandeling van veneuze trombo-embolie en secundaire preventie van arteriële trombose. Alphen aan den Rijn: Van Zuiden Communications; 2008.

20. Dutch Society for Internal Medicine: Guideline antithrombotic therapy. https://www.nvk.nl/Portals/0/richtlijnen/Trombo%20Embolie/Richtlijn%20Antitrombotisch%20beleid_def.pdf. Accessed February 24 2017.
21. Azar AJ, Cannegieter SC, Deckers JW, Briet E, van Bergen PF, Jonker JJ, et al. Optimal intensity of oral anticoagulant therapy after myocardial infarction. J Am Coll Cardiol. 1996;27(6):1349-55.
22. Oake N, Jennings A, Forster AJ, Fergusson D, Doucette S, van Walraven C. Anticoagulation intensity and outcomes among patients prescribed oral anticoagulant therapy: a systematic review and meta-analysis. CMAJ. 2008;179(3):235-44.
23. Bjorck F, Renlund H, Lip GY, Wester P, Svensson PJ, Sjalander A. Outcomes in a Warfarin-Treated Population With Atrial Fibrillation. JAMA Cardiol. 2016;1(2):172-80.
24. Rosendaal FR, Cannegieter SC, Meer FJ, Briët E. A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost. 1993;69(3):236-9.
25. De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, et al. Vitamin K antagonists in heart disease: current status and perspectives (Section III). Position paper of the ESC Working Group on Thrombosis--Task Force on Anticoagulants in Heart Disease. Thromb Haemost. 2013;110(6):1087-107.
26. Federation of Dutch Thrombosis Services. Summary annual medical reports 2015. Available at: https://s3.eu-central-1.amazonaws.com/storage.topsite.nl/fnt.nl/uploads/docs/jaarverslagen/Medisch_Jaarverslag_2015.pdf. Accessed February 24 2017.
27. Heneghan CJ, Garcia-Alamino JM, Spencer EA, Ward AM, Perera R, Bankhead C, et al. Self-monitoring and self-management of oral anticoagulation. Cochrane Database Syst Rev. 2016;7:CD003839.
28. Chai-Adisaksopha C, Crowther M, Isayama T, Lim W. The impact of bleeding complications in patients receiving target-specific oral anticoagulants: a systematic review and meta-analysis. Blood. 2014;124(15):2450-8.
29. Levy JH, Spyropoulos AC, Samama CM, Douketis J. Direct oral anticoagulants: new drugs and new concepts. JACC Cardiovasc Interv. 2014;7(12):1333-51.
30. Sardar P, Chatterjee S, Lavie CJ, Giri JS, Ghosh J, Mukherjee D, et al. Risk of major bleeding in different indications for new oral anticoagulants: insights from a meta-analysis of approved dosages from 50 randomized trials. Int J Cardiol. 2015;179:279-87.
31. Hansson PO, Sorbo J, Eriksson H. Recurrent venous thromboembolism after deep vein thrombosis: incidence and risk factors. Arch Intern Med. 2000;160(6):769-74.
32. Becattini C, Vedovati MC, Ageno W, Dentali F, Agnelli G. Incidence of arterial cardiovascular events after venous thromboembolism: a systematic review and a meta-analysis. J Thromb Haemost. 2010;8(5):891-7.
33. Lind C, Flinterman LE, Enga KF, Severinsen MT, Kristensen SR, Braekkan SK, et al. Impact of incident venous thromboembolism on risk of arterial thrombotic diseases. Circulation. 2014;129(8):855-63.
34. Rinde LB, Lind C, Smabrekke B, Njolstad I, Mathiesen EB, Wilsgaard T, et al. Impact of incident myocardial infarction on the risk of venous thromboembolism: the Tromso Study. J Thromb Haemost. 2016;14(6):1183-91.
35. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, et al. Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The Heart and Estrogen/progestin Replacement Study. Ann Intern Med. 2000;132(9):689-96.
36. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360(18):1851-61.
37. Pai M, Evans NS, Shah SJ, Green D, Cook D, Crowther MA. Statins in the prevention of venous thromboembolism: a meta-analysis of observational studies. Thromb Res. 2011;128(5):422-30.

38. van Schouwenburg IM, Mahmoodi BK, Gansevoort RT, Muntinghe FL, Dullaart RP, Kluin-Nelemans HC, et al. Lipid levels do not influence the risk of venous thromboembolism. Results of a population-based cohort study. Thromb Haemost. 2012;108(5):923-9.
39. Rahimi K, Bhala N, Kamphuisen P, Emberson J, Biere-Rafi S, Krane V, et al. Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. PLoS Med. 2012;9(9):e1001310.
40. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. Circulation. 2013;127(2):251-7.

CHAPTER 2

Agreement between Coaguchek XS and STA-R evolution (Hepato Quick) INR results depends on the level of INR

Thrombosis Research. 2015;136:652-7

Joseph S. Biedermann[1,2], Frank W.G. Leebeek[1], Peter N. Buhre[2], Sacha de Lathouder[2,3], Jan P.F. Barends[2], Moniek P.M. de Maat[1], Felix J.M. van der Meer[4], Marieke J.H.A. Kruip[1,2]

[1] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[2] Star-Medical Diagnostic Center, Rotterdam, the Netherlands
[3] Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, the Netherlands
[4] Department of Thrombosis and Hemostasis, Leiden University Medical Center, the Netherlands

ABSTRACT

Introduction

Introducing point-of-care (POC) INR measurement to monitor anticoagulant therapy may be beneficial for both patients and anticoagulation clinics. However, agreement between POC and laboratory INR results is still unclear, especially at sub- and supratherapeutic levels. Therefore we investigated the analytical and clinical agreement between POC INR results of the CoaguChek XS and laboratory INR results of the STA-R Evolution.

Materials and Methods

Paired POC and laboratory INR results were obtained and analyzed in 3257 patients aged 18-104 years between August 2008 and March 2014.

Results

Mean difference between POC and laboratory results ranged from -0.18 (95%CI -0.20;-0.16) INR point for POC results 2.0-3.0, up to 1.14 (95%CI 0.87;1.42) INR point for POC results 7.1-8.0. In the therapeutic range (POC INR 2.0-4.0), mean difference between POC and laboratory results was -0.13 (95%CI -0.15;-0.12) INR point. At subtherapeutic (POC INR <2.0) and supratherapeutic (POC INR >4.0) INR levels, mean differences were -0.13 (95%CI -0.15;-0.11) and 0.72 (95%CI 0.63;0.80) INR point, respectively. Clinical agreement regarding therapeutic range was present in 92.0% (POC within range), 67.7% (POC below range) and 87.6% (POC above range) of the paired measurements. We observed ≥15% INR difference between the POC and laboratory result in 14.8% (POC INR 2.0-4.0), 17.0% (POC INR<2.0) and 47.8% (POC INR >4.0) of the paired measurements.

Conclusions

POC and laboratory INR results were strongly correlated within the therapeutic range and differences between results become larger with increasing INR. Clinical disagreement between laboratory and POC results occurs often at both sub- and supratherapeutic INR levels.

INTRODUCTION

Anticoagulant therapy with vitamin K antagonists (VKA) is monitored by measuring the International Normalized Ratio (INR). The INR is a ratio between the prothrombin time measured in the patients' blood sample and the mean normal prothrombin time, corrected for the sensitivity of the used thromboplastin reagent (1). The required VKA dose to achieve or maintain a therapeutic INR can vary considerably since INR levels fluctuate during treatment and are sensitive to numerous endogenous and exogenous factors (2). Regular INR measurement and dose adjustment of VKA is important for treatment quality since overanticoagulated patients have an increased risk of bleeding complications, while underanticoagulated patients have an increased risk of thrombotic events (3,4).

Traditionally, the INR is measured in a laboratory in a venous blood sample. However, point-of-care (POC) devices have become available that make it possible to measure the INR directly in a single drop of capillary blood. These devices do not require venous blood sampling, which can be problematic, especially in frail elderly patients. With POC INR measurement patients can self-monitor (dosed by the physician or anticoagulation clinic) and even self-manage (self-dosing) VKA treatment. Moreover, INR self-monitoring using POC devices is associated with increased patient satisfaction and quality of life compared to routine care by anticoagulation clinics, without increasing the risk of bleeding or thrombotic events (5). Although several papers have been published discussing the accuracy and reproducibility of these devices, it is difficult to extrapolate the results when other laboratory methods or POC devices are used (6). Furthermore, agreement between POC and laboratory INR results at sub- or supratherapeutic INR levels is not well established. As POC INR measurement may offer advantages for both patients and anticoagulation clinics, knowledge of systematic differences between POC and laboratory results is crucial. Moreover, differences between POC and laboratory results could affect treatment quality like time in therapeutic range since dose adjustment protocols are still based on laboratory INR results. Therefore, we investigated the level of analytical and clinical agreement between POC INR results measured on the CoaguChek XS system and laboratory INR results measured on the STA-R Evolution in a large population of over 3000 patients on long-term anticoagulant treatment.

MATERIALS AND METHODS

Study population

All patients aged ≥ 18 years were included in whom a paired POC and laboratory INR measurement had been performed between August 2008 and March 2014 at the anticoagulation clinic of the STAR-Medical Diagnostic Center (Rotterdam, the Netherlands). In case multiple paired measurements had been performed in a patient, only the first pair was included, since patient-bound factors influencing the agreement between POC and laboratory results could not be excluded. Trained nurses of the clinic had obtained simultaneous capillary and venous blood samples, for POC and laboratory INR measurement respectively, in two different ways. Firstly, Point-of-care INR measurement, immediately followed by venous blood sampling for laboratory INR measurement, was performed in all patients eligible for self-monitoring between August 2008 and July 2013. Secondly, since 29 May 2013, all non-self-monitoring patients of one of the divisions managed by the Star-medical diagnostic center were from then on monitored using only the CoaguChek XS system. An additional venous sample for laboratory INR measurement was taken directly in all patients in case a POC INR was ≥6.0. Finally, a venous sample was also obtained in patients with a POC INR <6.0, who had reported unstable INR results since the introduction of POC INR monitoring. The study protocol was in accordance with the Helsinki II declaration and approved by the Medical Board of the anticoagulation clinic of the Star-Medical Diagnostic Center. Due to the observational nature of our study, waiver of informed consent was acquired from the medical ethical commission of the Erasmus University medical center.

POC INR assessment

POC INR measurements were performed using the CoaguChek XS system (Roche Diagnostics, Mannheim, Germany). The CoaguChek XS system consists of the CoaguChek XS measuring system and the CoaguChek XS test strips and has a measuring range of 0.8-8.0 INR. The test strips contain a lyophilized reagent that consists of a human recombinant thromboplastin with an ISI of 1.01 and a peptide substrate. The test principle is based on the conversion of an electrochemical signal generated by thrombin-mediated cleavage of this peptide substrate. All different lots of test strips used during the study were approved for use and validated by the Dutch Reference Laboratory for Anticoagulant Control (RELAC) to exclude lot-to-lot variation (7). In compliance with the manufacturer's instructions, one drop of capillary whole blood was applied to the test strip immediately after a finger stick procedure performed on the 3rd or 4th finger of the non-dominant hand. In terms of reproducibility, we found a

coefficient of variation (CV) of 2.9% in a validation study, which is consistent with CVs of 1.4-5.2% described in prior studies (6).

Laboratory INR assessment

For the laboratory INR measurement, approximately 2.7 ml blood was drawn by venipuncture into a BD Vacutainer® 0.109M (3.2%) Sodium Citrate blood collection tube. Blood samples were centrifuged at room temperature for 8 minutes at 2369x*g*. INR was determined using the STA Hepato Quick reagent on the STA-R Evolution automated coagulation system (Diagnostica Stago S.A.S, Asnières sur Seine, France), which uses a mechanical end-point detection method and can measure INR levels up to 12.5. The STA Hepato Quick reagent is a tissue-extract thromboplastin with an ISI of approximately 0.9 that uses an Owren-based method to determine the prothrombin time. ISI and mean normal prothrombin times were used according to the recommendations of RELAC after calibration for each lot number Hepato Quick that was used.

Assessment of clinical agreement

We interpreted INR results according to the patients' therapeutic range. In accordance with the guideline of the Dutch Federation of Anticoagulation Clinics, two target ranges are used in the Netherlands, which differ slightly from international guidelines (8). For each target range a somewhat wider acceptable therapeutic range is used. In the low intensity group, the target INR is 2.5–3.5 and the therapeutic range is between 2.0-3.5. In the high intensity group, the target INR is 3.0–4.0 and the therapeutic range is between 2.5-4.0. For patients with deviant target ranges, there is no difference between target and therapeutic range. We considered clinical agreement present if both the POC and laboratory result was either below, within or above the therapeutic range. Additionally, we determined paired measurements with a clinically relevant difference between the POC and laboratory result according to the definition by Poller et al. (≥15% INR difference) (9).

Statistical analysis

Analytical agreement between POC and laboratory results was analyzed using linear regression analysis and Bland-Altman Plots (10). Since non-linearity can occur when comparing INR results of systems using different thromboplastin reagents, orthogonal regression analysis was used to fit the data according to Tomenson's model using the Hepato Quick INR measurement as the reference system (11). Pearson's correlation coefficient was determined to assess the correlation between POC and laboratory results. A paired T-test was used to determine the mean INR difference between POC

and laboratory results, both overall and also for different POC INR cut-off values. All statistical tests were two-tailed and P-values <0.05 were considered significant. Analyses were performed using SPSS version 21.0 (IBM Corp. Armonk, NY, USA).

RESULTS

Between August 2008 and March 2014, paired POC and laboratory INR measurements had been performed in 3257 patients aged 18-104 years. Of these measurements, 2567 (78.8%) paired measurements were performed in self-monitoring candidates. The other 690 (22.2%) paired measurements were performed in patients who were monitored by the anticoagulation clinic using the CoaguChek XS system. Patient characteristics at moment of sampling are shown in table 1. In 326/3257 (10.0%) paired measurements, POC INR results >8.0 were found, which were analyzed separately, since they exceed the upper measurement level of the CoaguChek XS. Therefore, 2931 paired POC and laboratory INR results were included for final analyses.

Analytical agreement between POC and laboratory INR

A Bland-Altman plot showing the agreement between POC and laboratory INR results is shown in figure 1. Overall, there was a significant and strong correlation between the CoaguChek XS and STA-R Evolution using Hepato Quick (R=0.901, p<0.001). INR differences between POC and laboratory results are shown in table 2 and figure 2 for different POC INR cut-off levels. In the therapeutic range (POC INR 2.0-4.0, n=2092) mean difference between the POC and laboratory result was -0.13 (95%CI -0.15 to -0.12) INR point. At subtherapeutic INR levels (POC INR <2.0), mean difference between results was -0.13 (95%CI -0.15 to -0.11) INR point. At supratherapeutic INR levels (POC INR >4,0), mean difference between POC and laboratory results was 0.72 (95%CI 0.63 to 0.80) INR point. Calibration according to Tomenson, slightly improved the agreement between POC and laboratory INR results with a correlation coefficient of 0.903 between the adjusted POC and laboratory INR result and 95% limits of agreement of -1.34 to 1.03

Clinical agreement between POC and laboratory INR

Clinical agreement between POC and laboratory INR results is shown in table 3 and the proportion of paired measurements with 15% or more difference between results is shown in figure 3 for different POC INR cut-off values. In the therapeutic range (POC INR 2.0-4.0, n=2092), we observed a difference of 15% or more between results in 309/2092 (14.8%) paired measurements. Below the therapeutic range (POC INR <2.0,

n=320) 30/230 (17.0%) of the paired measurements had a difference of 15% or more between the POC and laboratory result. At supratherapeutic INR levels (POC >4.0, n=609), we found a difference of 15% or more in 291/609 (47.8%) paired measurements.

Table 1. Patient characteristics (n=3257)

Age, years	66.6 (18-104) *
Sex	
Male, n (%)	1887 (57.9)
Vitamin K antagonist	
Acenocoumarol, n (%)	3037 (93.2)
Phenprocoumon, n (%)	220 (6.8)
Treatment indication	
Atrial fibrillation, n (%)	1779 (54.6)
Venous thrombosis, n (%)	538 (16.5)
Heart valve replacement, n(%)	312 (9.6)
Other, n (%)	628 (19.3)
Treatment duration, years	2.0 [0.3-6.9] †
Therapeutic range, n (%)	
2.0-3.5	2469 (75.8)
2.5-4.0	701 (21.5)
Other, n (%)	87 (2.7)

Data are mean (range) * or median [interquartile-range] †.

Table 2. INR difference between POC and laboratory results by POC IN

POC INR	n	Mean POC	Mean lab	Mean difference (95% CI)
1.0-1.9	230	1.70	1.82	-0.13 (-0.15 to -0.11)
2.0-3.0	1345	2.55	2.72	-0.18 (-0.20 to -0.16)
3.1-4.0	747	3.48	3.53	-0.06 (-0.08 to -0.03)
4.1-5.0	193	4.41	4.29	0.12 (0.05 to 0.19)
5.1-6.0	86	5.70	5.06	0.64 (0.51 to 0.78)
6.1-7.0	188	6.47	5.43	1.05 (0.94 to 1.16)
7.1-8.0	142	7.66	6.52	1.14 (0.87 to 1.42)

POC = Point-of-care. A negative mean difference indicates that POC results are lower than laboratory results, a positive difference indicate that POC results are higher than laboratory results.

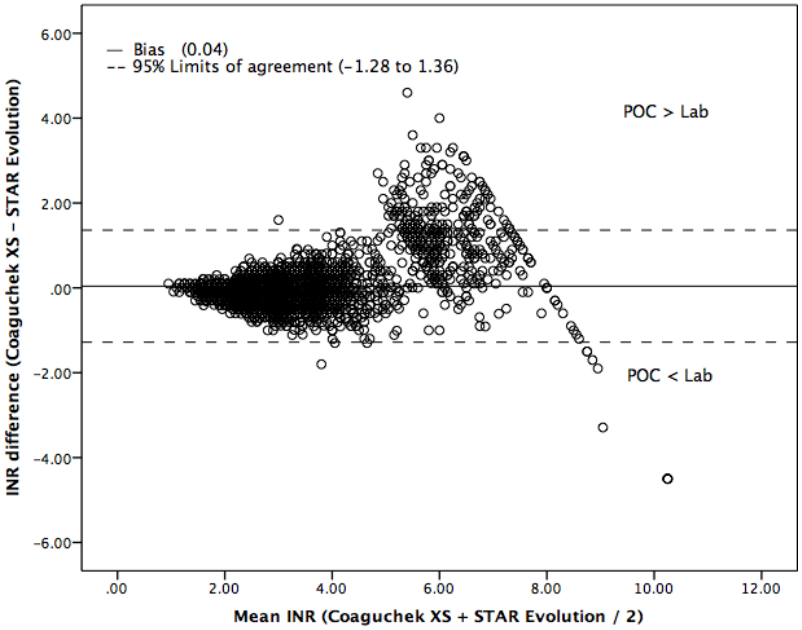


Figure 1. Bland-Altman plot of the difference between POC and laboratory results. Bland-Altman plot showing the difference in INR between POC and laboratory results against the average INR (n=2931). The dashed lines indicate the 95% limits of agreement (mean ± 1.96 SD) between test results.

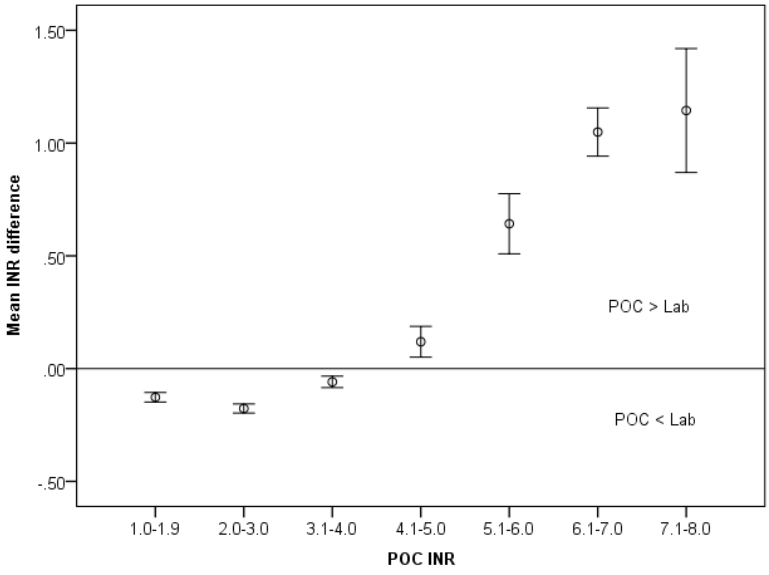


Figure 2. Mean difference between POC and laboratory results stratified by POC INR. Error bars represent the 95% confidence interval of the difference in INR between POC and laboratory results (n=2931).

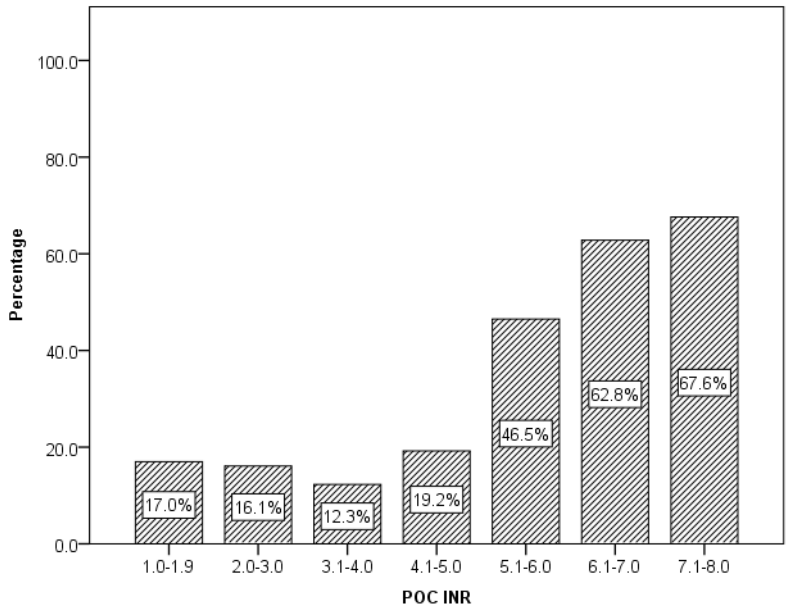


Figure 3. Fraction of paired INR results with 15% difference in INR or more. Bars indicate the fraction of paired measurements with a clinically significant difference (≥ 15% or more) between POC and laboratory results.

INR results >8.0

Of the 326 POC measured INR's >8.0, 83/326 (25.5%) of the laboratory results were also >8.0, 58/326 (17.8%) were between 7.1-8.0, 99/326 (30.4%) were between 6.1-7.0 and 64/326 (19.6%) were between 5.1-6.0. In 22/326 (6.7%) the laboratory result was 5.0 or lower.

DISCUSSION

The aim of our study was to investigate the analytical and clinical agreement between POC INR results of the CoaguChek XS and laboratory INR results in a broad INR range, in order to assess, in a real world setting, whether POC INR measurement can replace laboratory INR measurement of venous samples. We analyzed paired measurements in over 3000 patients and observed that the CoaguChek XS underestimated the INR below and within the therapeutic range (≤INR 4.0). In addition, above the therapeutic range (INR >4.0), the CoaguChek XS increasingly overestimated the INR with increasing INR levels compared to the STA-R Evolution using Hepato Quick.

Only a few studies have compared CoaguChek XS INR results with results of the STA-R Evolution (12,13). Our results are in line with earlier observations by Christensen et al. who found lower average INR results in a small study of 141 measurements on the CoaguChek XS compared to the STA-R Evolution, most of which were within the therapeutic range (12). Interestingly, Hur et al. found higher average INR results on the CoaguChek XS compared to the STA-R Evolution in 118 paired measurements of which 94% had a result of 3.0 or lower on the Coaguchek XS (13). This opposite finding compared to ours can be explained due to the difference in the thromboplastin reagent used for the INR measurement on the STA-R Evolution, since differences in sensitivity for circulating clotting factors have been described between recombinant and tissue-extract thromboplastin reagents (14). Both in our study and in the study by Christensen et al. a tissue-extract thromboplastin (Hepato Quick and SPA 50 respectively) was used on the STA-R Evolution, whereas in the study by Hur et al. the recombinant STA-Neoplastin was used. Furthermore, similar patterns in INR difference have been described when evaluating agreement between laboratory methods using recombinant versus tissue-extract thromboplastins (15,16). These findings, combined with our own, suggest that the agreement between methods can be estimated beforehand based on the sources of thromboplastin used in the investigated methods. Although venous whole blood was used for the CoaguChek measurement in Hur's study, this is not likely to explain the different results, since it has been shown that the bias between capillary and venous INR results on the CoaguChek XS system is negligible in the therapeutic range (17). Other possible explanations for the discrepancy are differences between study populations and the limited number of measurements included in the analyses. Although the mean INR difference between POC and laboratory results in our study was relatively small within or around the therapeutic range, even these small differences in clinically important INR ranges can significantly influence dosing decisions (18). This was illustrated by the fact that despite the small INR difference between both methods in the lower INR range, almost a third (32.3%) of the subtherapeutic POC results had a paired laboratory result within the therapeutic range, automatically resulting in a discrepancy in dosing-decision depending on which result is used to guide dosing. On the contrary, we observed increasing absolute INR differences at supratherapeutic levels and especially at POC INR levels ≥6.0 (n=369), i.e. a difference of 15% or more in over half (64.0%) of the paired samples. This was even more pronounced in the POC INR>8.0, of which only 25% of the paired laboratory INR results were also above 8.0. In this setting, dose reductions based on the POC INR result would often be excessive. However, it is important to emphasize that only plasma samples of patients with an INR between 1.5 and 4.5 may be used for INR calibration according to recommendations from the World Health Organization. Therefore the INR is only strictly valid up to an

INR of 4.5 regardless of which method is used. Since there is no absolutely true INR and it is not known which INR result is best to dose on, one could only speculate about the clinical consequences if patients would be routinely dosed on POC INR results. Besides, all data on the efficacy and safety of VKA and the determination of the target ranges are based on studies in which a laboratory INR was assessed and guided dosing. Future studies should determine the outcome of introduction of POC INR measurement in patients managed by physicians or anticoagulation clinics regarding the quality of treatment with VKA, since most trials on this subject have been performed in well-functioning patients capable of self-monitoring (19).

A drawback of our study is that we compared INR results using only one type of point-of-care device and with only Hepato Quick as reagent on the STA-R Evolution. Therefore, the differences between POC and laboratory results found in our study have to be interpreted prudently, especially when comparing INR results using other point-of-care devices or when a recombinant thromboplastin reagent, such as Innovin, is used to determine the laboratory INR result. In general, clinicians should be aware of potential INR differences when comparing systems using different thromboplastin sources, especially at supratherapeutic INR levels. Another limitation is the relatively small number of measurements in the higher INR range. However, although the number of supratherapeutic results was indeed relatively small compared to the number of results within the therapeutic range, 95% confidence intervals were robust, indicating adequate precision.

In conclusion, we found increasing INR differences between POC INR results of the CoaguChek XS compared to INR results of the STA-R Evolution using Hepato Quick with increasing INR levels in patients treated with vitamin K antagonists. Although absolute INR differences were small at (sub) therapeutic INR levels, dosing decisions would often be different, both at sub- and supra therapeutic INR levels.

REFERENCES

1. Poller L. International Normalized Ratios (INR): the first 20 years. J Thromb Haemost. 2004;2(6):849-60
2. Skov J, Bladbjerg EM, Rasmussen MA, Sidelmann JJ, Leppin A, Jespersen J. Genetic, Clinical and Behavioural Determinants of Vitamin K-Antagonist Dose - Explored Through Multivariable Modelling and Visualization. Basic Clin Pharmacol Toxicol. 2012;110(2):193–8
3. Hylek EM, Skates SJ, Sheehan MA, Singer DE. An Analysis of the Lowest Effective Intensity of Prophylactic Anticoagulation for Patients with Nonrheumatic Atrial Fibrillation. N Engl J Med. 1996;335(8):540-6
4. Hylek EM, Singer DE. Risk factors for intracranial hemorrhage in outpatients taking warfarin. Ann Intern Med. 1994;120(11):897-902.
5. Garcia-Alamino JM, Ward AM, Alonso-Coello P, Perera R, Bankhead C, Fitzmaurice D, et al. Self-monitoring and self-management of oral anticoagulation. Cochrane Database Syst Rev. 2010 Apr 14;(4):CD003839
6. Christensen TD, Larsen TB. Precision and accuracy of point-of-care testing coagulometers used for self-testing and self-management of oral anticoagulation therapy. J Thromb Haemost 2012;10(2):251–60.
7. van den Besselaar AM, Pequeriaux NC, Ebben M, van der Feest J, de Jong K, Ganzeboom MB, et al. Point-of-care monitoring of vitamin K-antagonists: validation of CoaguChek XS test strips with International Standard thromboplastin. J Clin Pathol. 2012;65(11):1031-5.
8. Federatie van Nederlandse Trombosediensten (2014) De kunst van het doseren, 3e herziene druk. [cited 2014 May]; Available: http://www.fnt.nl/media/docs/overig/de_kunst_van_doseren.pdf.
9. Poller L, Keown M, Ibrahim SA, van der Meer FJ, van den Besselaar AM, Tripodi A, et al. Quality assessment of CoaguChek point-of-care prothrombin time monitors: comparison of the European community-approved procedure and conventional external quality assessment. Clin Chem. 2006;52(10):1843-7.
10. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307–10.
11. Tomenson JA. A statistician's independent evaluation. In: van den Besselaar AMHP, Gralnick HR, Lewis SM, eds. *Thromboplastin Calibration and Oral Anticoagulant Control.* Boston, MA: Martinus Nijhoff Publishers; 1984:87–108.
12. Christensen, TD, Larsen TB, Jensen, C, Maegaard, M, Sørensen, B. International normalised ratio (INR) measured on the CoaguChek S and XS compared with the laboratory for determination of precision and accuracy. Thromb Haemost. 2009;101(3):563-9.
13. Hur M, Kim H, Park CM, La Gioia A, Choi SG, Choi JH et al. Comparison of international normalized ratio measurement between CoaguChek XS Plus and STA-R coagulation analyzers. Biomed Res Int. 2013;2013:213109.
14. Testa S, Morstabilini G, Fattorini A, Galli L, Denti N, D'Angelo A. Discrepant sensitivity of thromboplastin reagents to clotting factor levels explored by the prothrombin time in patients on stable oral anticoagulant treatment: impact on the international normalized ratio system. Haematologica. 2002;87(12):1265-73.
15. van den Besselaar AM, van Zanten AP, Brantjes HM, Elisen MG, van der Meer FJ, Poland DC, et al. Comparative study of blood collection tubes and thromboplastin reagents for correction of INR discrepancies: a proposal for maximum allowable magnesium contamination in sodium citrate anticoagulant solutions. Am J Clin Pathol. 2012;138(2):248-54
16. Remijn JA, Wildeboer B, van Suijlen JD, Adriaansen HJ. Recombinant Thromboplastins vs Tissue-Extract Thromboplastins in Patients on Unstable Oral Anticoagulant Therapy. Clin Chem. 2011;57(6):916-7.

17. Plesch W, van den Besselaar AM. Validation of the international normalized ratio (INR) in a new point-of-care system designed for home monitoring of oral anticoagulation therapy. Int J Lab Hematol. 2009;31(1):20–5.
18. Shermock KM, Connor, JT, Lavallee DC, Streif MB. Clinical decision-making as the basis for assessing agreement between measures of the International Normalized Ratio. J Thromb Haemost. 2009;7:87-93.
19. Heneghan C, Alonso-Coello P, Garcia-Alamino JM, Perera R, Meats E, Glasziou P. Self-monitoring of oral anticoagulation: a systematic review and meta-analysis. Lancet. 2006;367:404-11.

CHAPTER 3

Point-of-care testing and INR within-subject variation in patients receiving a constant dose of vitamin K antagonist

Thrombosis and Haemostasis. 2015;114:1260-7

Antonius M. van den Besselaar[1]; Joseph S. Biedermann[2,3]; Marieke J.H.A. Kruip[2,3]

[1] Department of Thrombosis and Haemostasis, Leiden University Medical Center, the Netherlands
[2] Department of Haematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[3] Star-Medical Diagnostic Center, Rotterdam, the Netherlands

SUMMARY

Many patients treated with vitamin K antagonists (VKA) determine their INR using point-of-care (POC) whole blood coagulation monitors. The primary aim of the present study was to assess the INR within-subject variation in self-testing patients receiving a constant dose of VKA. The second aim of the study was to derive INR imprecision goals for whole blood coagulation monitors. Analytical performance goals for INR measurement can be derived from the average biological within-subject variation. Fifty-six Thrombosis Centres in the Netherlands were invited to select self-testing patients who were receiving a constant dose of either acenocoumarol or phenprocoumon for at least six consecutive INR measurements. In each patient, the coefficient of variation (CV) of INRs was calculated. One Thrombosis Centre selected regular patients being monitored with a POC device by professional staff. Sixteen Dutch Thrombosis Centres provided results for 322 selected patients, all using the CoaguChek XS. The median within-subject CV in patients receiving acenocoumarol (10.2 %) was significantly higher than the median CV in patients receiving phenprocoumon (8.6 %) (P=0.001). The median CV in low-target intensity acenocoumarol self-testing patients (10.4 %) was similar to the median CV in regular patients monitored by professional staff (10.2 %). Desirable INR analytical imprecision goals for POC monitoring with CoaguChek XS in patients receiving either low-target intensity acenocoumarol or phenprocoumon were 5.1 % and 4.3 %, respectively. The approximate average value for the imprecision of the CoaguChek XS, i. e. 4 %, is in agreement with these goals.

INTRODUCTION

In the Netherlands, patients receiving long-term treatment with vitamin K-antagonists (VKA) are managed and controlled by Thrombosis Centres (1). Increasing numbers of patients are trained to determine their INR by themselves using a point-of-care whole-blood coagulation monitor. By the end of the year 2013, approximately 13% of all patients in the Netherlands were using whole blood coagulation monitors for INR self-testing at home (2). Also in other European countries and the United States large numbers of patients perform INR self-measurement (3,4). The quality of self-testing depends partly on the analytical performance of the whole blood monitor. By increasing the quality of INR measurements, the quality of treatment can potentially be further increased (5).

It is widely accepted that analytical performance goals of a laboratory method should be based on biological variation of the analyte. Fraser et al introduced the concept of minimum, desirable and optimum quality goals for imprecision and inaccuracy of laboratory methods and point-of-care testing (6,7). In previous studies, the biological within-subject variation of the INR was assessed using conventional laboratory determinations of the prothrombin time in plasma samples from patients who were stabilized on long-term treatment with VKA (8-11). It is well known that there are INR differences between assay systems. The reasons for discrepancies between INR results determined by point-of-care instruments and laboratory measurements are not fully understood (12). It is not known whether within-subject variation of INR determined by conventional laboratory methods is different from the within-subject variation of INR determined by point-of-care whole blood monitors.

The aim of the present study was to assess the INR within-subject variation in patients taking a constant dose of either acenocoumarol or phenprocoumon and who used a point-of-care whole blood coagulation monitor for INR self-testing. All self-testing patients used the same type of whole blood coagulation monitor, i.e. the CoaguChek XS (Roche Diagnostics, Mannheim, Germany). The second aim of the study was to derive the desirable imprecision goal for the whole blood coagulation monitor.

MATERIALS AND METHODS

All members of the Netherlands Federation of Thrombosis Centres were invited to participate in the study. At the time of the study there were 56 member centres. Recruitment of participants and collection of data started in February 2014 and was concluded in August 2014. Each centre was requested to select at least 24 patients from

the centre's total patient population according to criteria described below. Centres were instructed to review the patients' files in alphabetical order of their surnames. The selected patients data were provided anonymously to the authors for statistical analysis. Hence the data could not be traced back to the patients' identification and informed consent was not required. Patients received either acenocoumarol or phenprocoumon. Depending on the indication for treatment, one of two different therapeutic target ranges were applied, i.e. either INR 2.0-3.5 (low intensity) or INR 2.5-4.0 (high intensity). The target range in the Netherlands is set higher than the internationally advised target range to prevent inadequate anticoagulation, i.e. INR < 2.0 (13).

The following criteria were used for patient selection:
- VKA treatment period was 6 months or longer;
- Self-testing by the patient with a home whole blood INR monitor using capillary blood samples;
- After the initial treatment period of 6 months or longer, a period of constant dose of VKA was achieved during which at least six consecutive INRs were recorded;
- The interval between consecutive INR determinations was two weeks or longer;
- In the period of constant dose of VKA and at least six consecutive INR determinations, there were no changes in conditions that may influence the INR such as intercurrent diseases, invasive procedures, or starting/stopping other drugs interacting with VKA.

Four groups of selected patients were formed from all centres:
- Patients receiving acenocoumarol, low target intensity;
- Patients receiving acenocoumarol, high target intensity;
- Patients receiving phenprocoumon, low target intensity;
- Patients receiving phenprocoumon, high target intensity.

Each centre was requested to select at least six patients for each group. For a comparison between self-testing patients and patients regularly pricked by professional staff, one centre selected an additional group of 123 patients receiving acenocoumarol with low target intensity who were pricked and monitored with the CoaguChek XS Pro by professional staff. The latter group of patients is denoted as "regular" patients. The regular patients, apart from being pricked and tested by professionals, were selected with the same criteria as those for the self-testing patients. The CoaguChek XS Pro instrument has been developed by Roche Diagnostics (Mannheim, Germany) using the same measurement principle as the CoaguChek XS (14).
For each patient, we calculated the coefficient of variation (CV) of the INRs of the constant dose period. CV_T represents the median CV for a group of patients with the

same drug and target intensity. For calculation of the average biological within-subject variation (CV_B), we used the formula given by Lassen et al (8):

$$CV_B = \sqrt{(CV_T^2 - CV_A^2)}$$

CV_A is the mean analytical imprecision coefficient of variation of the capillary blood INR determination using the CoaguChek XS. We used CV_A = 4% which is an average value derived from previous reports (15-18).

The Kolmogorov-Smirnov test was used to test the null hypothesis that the CV data came from a normally distributed population. Pearson's correlation and Spearman's rank correlation were used to analyse the correlation between patient age and CV. Differences in CV between the above mentioned patient groups were analysed using Student's t-test and the Mann-Whitney test. *P* values of less than 0.05 were considered statistically significant. All statistical analyses were performed with SPSS 20 (IBM Corporation, Armonk, NY, USA) for Windows.

Time in therapeutic range was calculated using an approach in which the INR of an individual patient is gradually increasing or decreasing between two successive measurements (19). Person-time was allocated to the therapeutic range using linear interpolation between two successive INR measurements and summed over all patients.

RESULTS

Sixteen out of fifty-six Thrombosis Centres in the Netherlands responded and participated in the study. Each participating centre was requested to select patients according to the above-mentioned criteria. All selected patients used the CoaguChek XS for self-testing. The number of selected patients in each of the four groups varied from centre to centre. The total number of the patients in each group is given in Table 1. The numbers of patients in the low target intensity groups were greater than those in the high target intensity groups, because some Thrombosis Centres could hardly find patients with high intensity treatment. There was a wide range of patients' ages and treatment times prior to the period of constant dose. There was also a wide range in observation periods varying between 70 and 638 days. The midterm calendar month was determined for each patient. The major part of the selected self-testing patients (58.4 %) were observed mainly in the winter season (i.e., midterm calendar months December, January or February). Only 9.6% of the selected patients were observed mainly in the summer (i.e., midterm calendar months June, July or August).

Table 1. Basic characteristics of selected patients

Drug	INR Target	Self-testing	N	Male (%)	Age (years)		Treatment time prior to entry (months)	
					Mean	Range	Mean	Range
Acenocoumarol	low	yes	93	75.3	65.7	22-85	74.9	11-291
Acenocoumarol	high	yes	72	66.7	63.3	29-84	105.2	12-291
Phenprocoumon	low	yes	94	60.6	62.1	35-83	79.9	6-332
Phenprocoumon	high	yes	63	66.7	62.4	28-85	141.5	24-386
Acenocoumarol	low	no*	123	56.9	72.1	35-95	82.7	7-292

* All patients in this group were selected by a single centre. N = number of patients.

According to the selection criteria, the minimum number of INR's for each patient was 6 but some Thrombosis Centres found patients with more than 6 INR's in the period of constant dose (Table 2). For each patient, the mean INR in the period of constant dose was calculated. As expected, the mean INR's for patients in the high target intensity groups were higher than those in the low intensity groups (Table 2). The differences in mean INR's between low-target and high-target groups were statistically significant for both acenocoumarol (t-test, $P < 0.001$) and phenprocoumon (t-test, $P < 0.001$). For each patient, the CV of INR's in the period of constant dose was calculated. Histograms of CV's are shown in Figure 1. The null hypothesis of a normal distribution was not rejected (P=0.091 for high target phenprocoumon, P=0.200 for other patient groups). For each of the two VKA drugs, there was no significant difference in the mean CV's between low and high intensity treatment (t-test: P=0.650 for acenocoumarol; P=0.155 for phenprocoumon). For this reason the results of low and high target intensities were combined. The median CV was greater for patients receiving acenocoumarol (CV_T = 11.0%) than the median CV for patients receiving phenprocoumon (CV_T = 9.5%) (Table 2). The CV's were compared and the differences between the acenocoumarol (n = 165) and phenprocoumon (n=157) groups were significant (t-test: P=0.001; Mann-Whitney test: P=0.002). The median within-subject CV_B for patients receiving acenocoumarol and for patients receiving phenprocoumon was 10.2% and 8.6%, respectively. There was no correlation between the mean testing interval and the within-subject CV, either for acenocoumarol (n=165, Spearman's rho=0.047, P=0.550) or for phenprocoumon (n=157, Spearman's rho = 0.125, P=0.120).

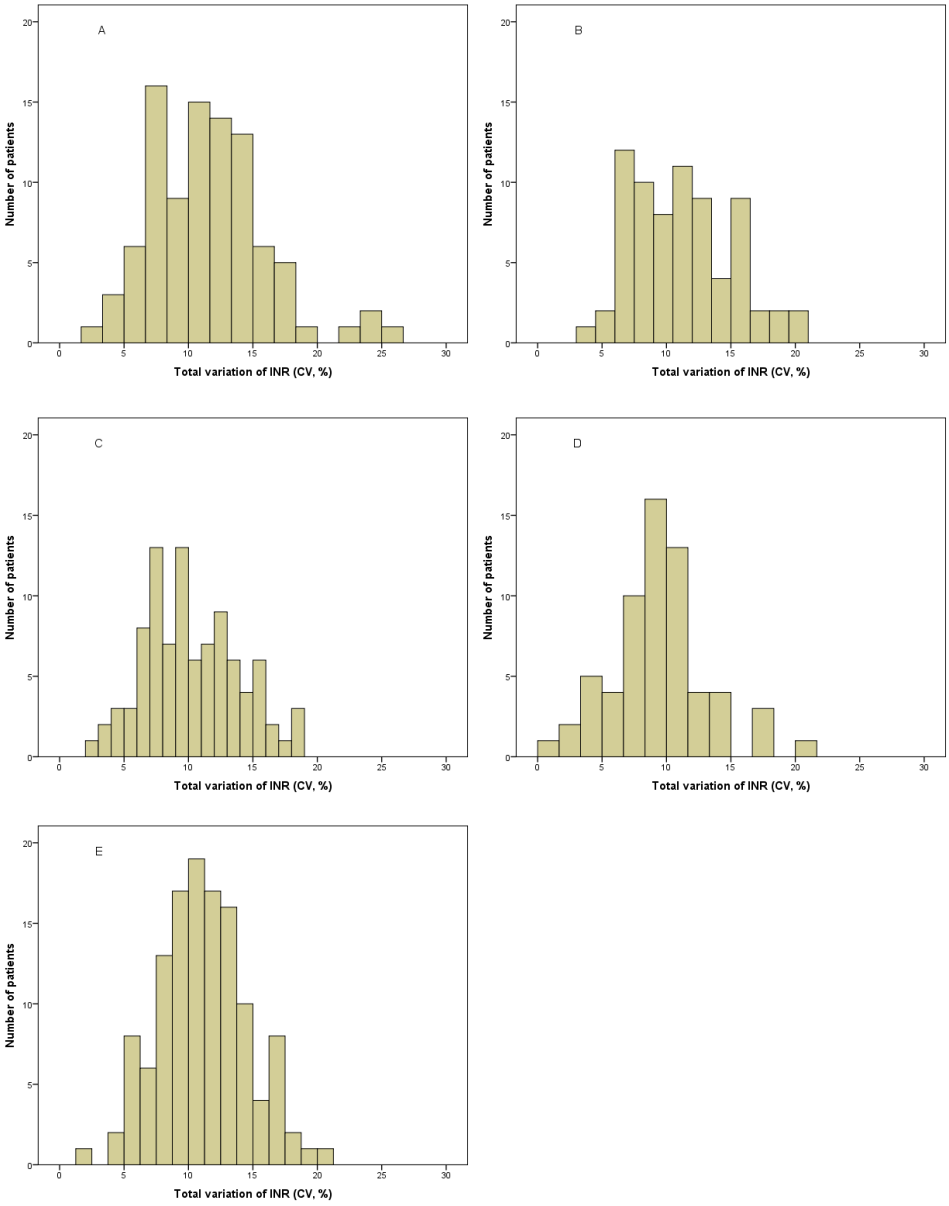


Figure 1. **Histogram of patients' INR coefficient of variation.** Panels A, B, C, and D refer to self-testing patients receiving acenocoumarol (low target intensity), acenocoumarol (high target intensity), phenprocoumon (low target intensity) and phenprocoumon (high target intensity), respectively. Panel E refers to patients receiving acenocoumarol (low target intensity) being pricked and monitored by professional staff.

There was a significant correlation between age and CV for patients receiving acenocoumarol (n=165, Spearman's rho = -0.202, *P*=0.009). The CV tended to lower values with increasing age (Figure 2). There was neither a significant correlation between age and CV for patients receiving phenprocoumon (n=157, Spearman's rho = -0.075, *P*=0.353), nor for regular patients receiving low-intensity acenocoumarol (n=123, Spearman's rho = -0.093, *P*=0.304).

We compared the group of self-testing patients receiving low-target acenocoumarol to a group of regular low-target acenocoumarol patients who were pricked and tested by professionals using the CoaguChek XS Pro. It should be noted that self-testing patients were instructed to perform the test and report the results every two weeks. The regular patients were less frequently pricked and tested by professionals. The frequency difference between self-testing and regular patients is reflected by the longer time interval between tests for the regular patients (Table 2). The mean CV of the self-testing patients was not statistically different from the mean CV of the regular patients pricked and tested by the professionals (t-test: *P*=0.409; Mann-Whitney test: *P*=0.690).

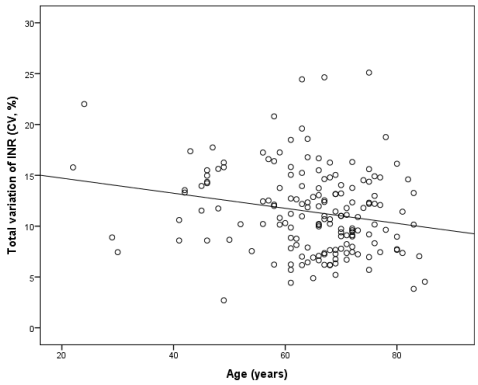


Figure 2. Scatter plot of total variation of INR in self-testing patients receiving acenocoumarol as a function of age (n=165, Spearman's rho = -0.202, *P*=0.009). The data of low target and high target intensity patients are shown, as well as a linear regression line.

Table 2. Variation of INR in selected patients

Drug	INR Target	Self-test	Number of INR measurements per patient		Mean interval between measurements (days)		Mean INR per patient		TTR (%)	CVT (%)	CVB (%)
			Mean	Range	Mean	Range	Mean	Range			
Acenocoumarol	low	yes	6.6	6-12	18.2	14-39	2.88	2.4-3.7	97.3	11.1	10.4
Acenocoumarol	high	yes	6.4	6-13	18.1	14-34	3.39	3.0-3.8	97.9	11.0	10.2
Phenprocoumon	low	yes	7.0	6-22	19.0	14-33	2.84	2.2-3.3	98.9	9.7	8.8
Phenprocoumon	high	yes	6.5	6-13	18.9	14-31	3.37	2.8-3.8	97.5	9.1	8.1
Acenocoumarol	low	no*	6.7	6-10	30.9	15-42	2.91	2.6-3.4	99.6	11.0	10.2

* All patients in this group were selected by a single centre. CVT is de median of total variation. CVB is the average biological within-subject variation. TTR is the mean time in therapeutic range.

DISCUSSION

The aim of the present study was to determine the overall within-subject variation of the INR in a sample of self-testing patients receiving a constant dose of VKA. The selection of the patients was entrusted with the Netherlands Thrombosis Centres. Apart from the selection criteria mentioned in the Materials and Methods, no restrictions were made with regard to indications for anticoagulation, comedication of antiplatelet agents, gender, time of year, and ethnicity. Although information about indications for anticoagulation was not provided, it is likely that all major indications were represented. Self-testing is primarily performed by patients who are receiving VKA for an indefinite length of time or lifelong. The relatively high percentage of male patients in our study (Table 1) might be explained by the fact that a greater proportion of male patients is associated with atrial fibrillation and artificial heart valves (2,3).
The majority of the selected patients were observed mainly in the winter season. When the Thrombosis Centres received the invitation to participate they probably were inclined to select patients observed in the most recent months, i.e., February, January, and December. Previous studies have shown that there were significant seasonal variations in the mean INR measurements, with the lowest values in summer and the highest values in autumn (20). Seasonal effects might have contributed to the within-

subject variation CV observed in the present study, but the study was not designed to assess the contribution of seasonal effects.

In the present study, we observed a wide range of within-subject CV's of INR's among patients receiving a constant dose of either acenocoumarol or phenprocoumon. The wide ranges are similar to those observed in stable patients monitored with a conventional laboratory prothrombin time system (9). Within-subject variation of the INR in patients receiving a constant dose of VKA is partially due to variation of vitamin K in the diet (21). It is not known whether the observed differences in CV between individual patients are systematic or time dependent. The Netherlands Thrombosis Centres use common guidelines for dosing and monitoring of their patients. For this reason it was justified to combine the patient data from various centres for analysis.

The patients included in the present study were all treated for six months or longer before their INRs were analysed. We did not observe a correlation between within-subject CV and the treatment time prior to the study observation period. In a systematic review and meta-analysis of self-monitoring, the time in therapeutic range improved and standard deviations decreased over time (22). The main part of improvements was observed in the first six months of intervention (22).

The total variation of the INR in patients on acenocoumarol was slightly, but significantly, greater than in patients receiving phenprocoumon (Table 2). This finding is in agreement with previous results in patients monitored with conventional laboratory prothrombin time systems (10,11,23). Greater within-subject variation of the INR in patients receiving acenocoumarol could be explained by the shorter half-life of acenocoumarol (i.e. compared to that of phenprocoumon) leading to greater variation of the drug concentration and of residual factor VII activity (24,25). It is interesting to note that the average within-subject variation in self-testing patients receiving acenocoumarol (CV_T = 10.2 – 10.4%, table 2) is similar or slightly lower than the average variation in steady-state patients receiving the same VKA monitored by a conventional laboratory method (CV_T = 10.5 – 10.9%) (9).

We observed a significant correlation between age and the total variation of the INR for self-testing patients receiving acenocoumarol (Figure 2). Age was not a selection criterium in our study and a wide range of ages between 22 and 85 years was obtained (Table 1). In contrast, we did not find a significant correlation in self-testing patients receiving phenprocoumon nor in regular patients receiving acenocoumarol with low-target intensity. We can only speculate why age might have an influence on the CV of the INR in self-testing patients receiving acenocoumarol. Better adherence to acenocoumarol treatment regimens among older patients might have been a factor. Other studies have shown that independent predictors of stable INR were age>70 years, male gender and the absence of heart failure (26).

Little is known about the imprecision of INR determined by patients using a home whole blood coagulation monitor. Estimating the precision of a whole blood coagulometer presents problems, as it is not possible to perform ordinary within-run or between-run estimations (5). In one report on the imprecision of the CoaguChek XS, it was stated that the CV was 5.92% at the start of determinations by patients and 5.16% at the final session (15). In subsequent reports, the values for imprecision of capillary blood INR by the CoaguChek XS were lower: 3.9-4.0% (16), 2.24% (17), 3.2% (18). The magnitude of the contribution by preanalytical errors to the total variation is uncertain. It is not known whether preanalytical errors are more prominent in self-testing patients as compared to professional staff. However, we did not observe a significant difference in CV_T between self-testing patients and patients pricked by professional staff (Table 2). This observation suggests that the imprecision, including preanalytical errors, is similar in both groups of patients. Self-testing patients may have achieved similar technical skills as professional staff.

Another factor which may contribute to the total within-subject variation is the between-lot variation of test strips. Minor differences between strip lots have been observed (27). We did not control the switching from one lot of strip lot to another in the present study. It cannot be excluded that patients changed test strip lots during the period of constant dose of anticoagulant.

In conclusion, our results have important consequences for the analytical performance goals of the INR determination by means of whole blood point-of-care coagulation monitors like the CoaguChek XS. For patients receiving acenocoumarol, the desirable imprecision goal according to Fraser et al (3,4) is $0.50 \times CV_B = 5.1\%$. Likewise, the desirable imprecision goal for patients receiving phenprocoumon is 4.3%. The approximate average value for the imprecision of the CoaguChek XS reported in the literature, i.e. 4%, is in agreement with these goals.

REFERENCES

1. Van Geest-Daalderop JH, Sturk A, Levi M, Adriaansen HJ. Extent and quality of anticoagulation treatment with coumarin derivatives by the Dutch Thrombosis Services. Ned Tijdschr Geneeskd 2004;148:730-5.
2. Netherlands Federation of Thrombosis Services. Summary medical annual reports 2013. Available from http://www.fnt.nl/.
3. Le Heuzey JY, Ammentorp B, Darius H, De Caterina R, Schilling RJ, Schmitt J, et al. Differences among western European countries in anticoagulation management of atrial fibrillation. Data from the PREFER IN AF registry. Thromb Haemost 2014;111(5):833-41.
4. Ansell J. Point-of-care patient self-monitoring of oral vitamin K antagonist therapy. J Thromb Thrombolysis 2013;35(3):339-41.
5. Christensen TD, Larsen TB. Precision and accuracy of point-of-care testing coagulometers used for self-testing and self-management of oral anticoagulation therapy. J Thromb Haemost 2012;10(2):251-60.
6. Fraser CG, Hyltoft Petersen P, Libeer JC, Rios C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997;34(Pt 1):8-12.
7. Fraser CG. Optimal analytical performance for point-of-care testing. Clin Chim Acta 2001;307(1-2):37-43.
8. Lassen JF, Brandslund I, Antonsen S. International Normalized Ratio for prothrombin times in patients taking oral anticoagulants: critical difference and probability of significant change in consecutive measurements. Clin Chem 1995;41(3):444-7.
9. Van Geest-Daalderop JH, Péquériaux NC, van den Besselaar AM. Variability of INR in patients on stable long-term treatment with phenprocoumon and acenocoumarol and implications for analytical quality requirements. Thromb Haemost 2009;102(3):588-92.
10. Van Geest-Daalderop JH, Kraaijenhagen RJ, van der Meer FJ, van den Besselaar AM. Intraindividual variation of the International Normalized Ratio in patients monitored with a recombinant human thromboplastin. J Thromb Haemost 2010;8(7):1641-2.
11. Van den Besselaar AM, Fogar P, Pengo V, Palareti G, Braham S, Moia M, et al. Biological variation of INR in stable patients on long-term anticoagulation with warfarin. Thromb Res 2012;130(3):535-7.
12. Sølvik UØ, Røraas TH, Petersen PH, Stavelin AV, Monsen G, Sandberg S. Effect of coagulation factors on discrepancies in International Normalized Ratio results between instruments. Clin Chem Lab Med 2012;50(9):1611-20.
13. Bezemer ID, Roemer WH, Penning-van Beest FJ, van Eekelen E, Kramer MH. INR control calculation: comparison of Dutch and international methods. Neth J Med 2013;71(4):194-8.
14. Cotte J, Lacroix G, D'Aranda E, Kaiser E, Meaudre E. Management of traumatic coagulopathy during long-distance medical evacuation: utility of the Coaguchek(®) XS pro [article in French]. Ann Fr Anesth Reanim 2013;32(2):122-3.
15. Braun S, Watzke H, Hasenkam JM, Schwab M. Wolf T, Dovifat C, et al. Performance evaluation of the new CoaguChek XS system compared with the established CoaguChek system by patients experienced in INR-self management. Thromb Haemost 2007;97(2):310-4.
16. Plesch W, Wolf T, Breitenbeck N, Dikkeschei LD, Cervero A, Perez PL, et al. Results of the performance verification of the CoaguChek XS system. Thromb Res 2008;123(2):381-9.
17. Wieloch M, Hillarp A, Strandberg K, Nilsson C, Svensson PJ. Comparison and evaluation of a point-of-care device (CoaguChek XS) to Owren-type prothrombin time assay for monitoring of oral anticoagulant therapy with warfarin. Thromb Res 2009;124(3):344-8.
18. Sølvik UØ, Petersen PH, Monsen G, Stavelin AV, Sandberg S. Discrepancies in International Normalized Ratio results between instruments: a model to split the variation into subcomponents. Clin Chem 2010;56(10):1618-26.
19. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E. A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost 1993;69(3):236-39.

20. Salobir B, Šabovič M, Peternel P. Intensity of long-term treatment with warfarin is influenced by seasonal variations. Pathophysiol Haemost Thromb 2002;32(4):151-4.
21. Karlson B, Leijd B, Hellstrom K. On the influence of vitamin K-rich vegetables and wine on the effectiveness of warfarin treatment. Acta Med Scand. 1986;220(4):347-50.
22. Heneghan C, Ward A, Perera R, Self-Monitoring Trialist Collaboration, Bankhead C, Fuller A, et al. Self-monitoring of oral anticoagulation: systematic review and meta-analysis of individual patient data. Lancet 2012;379(9813):322-34.
23. Fihn SD, Gadisseur AA, Pasterkamp E, van der Meer FJ, Breukink-Engbers WG, Geven-Boere LM, et al. Comparison of control and stability of oral anticoagulant therapy using acenocoumarol versus phenprocoumon. Thromb Haemost 2003;90(2):260-6.
24. Thijssen HH, Hamulyák K, Willigers H. 4-Hydroxycoumarin oral anticoagulants: pharmacokinetics-response relationship. Thromb Haemost 1988;60(1):35-8.
25. Van Geest-Daalderop JH, Hutten BA, Péquériaux NC, Haas FJ, Levi M, Sturk A. The influence on INRs and coagulation factors of the time span between blood sample collection and intake of phenprocoumon or acenocoumarol: consequences for the assessment of the dose. Thromb Haemost 2007;98(4):738-46.
26. Witt DM, Delate T, Clark NP, Martell C, Tran T, Crowther MA, et al. Twelve-month outcomes and predictors of very stable INR control in prevalent warfarin users. J Thromb Haemost 2010;8(4):744-9.
27. Van den Besselaar AM, Péquériaux NC, Ebben M, van der Feest J, de Jong K, Ganzeboom MB, et al. Point-of-care monitoring of vitamin K-antagonists: validation of CoaguChek XS test strips with international standard thromboplastin. J Clin Pathol 2012;65(11):1031-5.

CHAPTER 4

Impact of point-of-care INR monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: A cohort study

Journal of Thrombosis and Haemostasis. 2016;14:695-703

Joseph S. Biedermann[1,2], Nienke van Rein[3,4], Antonius M.H.P. van den Besselaar[3], Peter N. Buhre [2], Moniek P.M. de Maat[1], Felix J.M. van der Meer[3], Frank W.G. Leebeek[1], Marieke J.H.A. Kruip[1,2]

[1] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[2] Star-Medical Diagnostic Center, Rotterdam, the Netherlands
[3] Department of Thrombosis and Hemostasis, Leiden University Medical Center, the Netherlands
[4] Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, the Netherlands

ABSTRACT

Background

Point-of-care (POC) international normalized ratio (INR) monitoring by healthcare professionals could eliminate the need for venous blood sampling in non-self-monitoring (NSM) patients on vitamin K antagonists (VKA). However, few studies have investigated the impact of POC INR monitoring on the quality of treatment in these patients and real-world data on this issue are lacking.

Objectives

To investigate the safety, efficacy and quality of anticoagulant control during POC INR monitoring as compared with laboratory INR monitoring in NSM patients.

Methods

We performed a retrospective cohort study using data from the anticoagulation clinic of the Star-Medical Diagnostic Center (Rotterdam, the Netherlands). Patients who received treatment with VKA between 29 May 2012 and 29 May 2014 were eligible. Percentage of time in therapeutic range (TTR) and incidence rates of major clinical events (all-cause mortality, hospitalization, major bleeding and ischemic stroke) were compared for the year before and year after introduction of POC monitoring. Cox proportional hazard models were used to estimate hazard ratios and 95% confidence intervals for major clinical events between exposure groups.

Results

In total, 1973 patients during the 1-year laboratory-monitoring observation period and 1959 patients during the 1-year POC-monitoring observation period were included. Median TTR was significantly lower during POC monitoring (77.9%; 95% CI, 67.2-87.4) than during laboratory INR monitoring (81.0%; 95% CI, 71.1-90.5). Adjusted hazard ratios for major clinical events were all around unity.

Conclusions

Although associated with lower TTR, POC INR monitoring is a safe and effective alternative to laboratory INR monitoring in NSM patients on VKA

INTRODUCTION

The goal of anticoagulant therapy with vitamin K antagonists (VKA) is to reduce the risk of thrombotic events while keeping the treatment-related risk of bleeding complications as low as possible. To achieve this goal, the International Normalized Ratio (INR) must be maintained within a narrow therapeutic range, which requires INR monitoring and dose adjustment of VKA (1). During VKA treatment, the most commonly used indicator for quality of anticoagulation control is the percentage of time in therapeutic range (TTR): a low TTR is associated with an increased risk of major bleeding, ischemic stroke, and mortality (2-4). In 2013, more than 450,000 patients were treated with VKA in the Netherlands, of whom 87% were monitored by professionals at anticoagulation clinics and 13% performed INR self-monitoring using point-of-care (POC) devices (5). An advantage of using a POC device is the instantaneous measurement of the INR in just a single drop of capillary blood. Furthermore, POC devices enable patients to participate more actively in their VKA treatment, either through self-monitoring (measurement by the patient and dosing by a physician) or even through self-management (self-measurement and self-dosing) (6). The INR of most of the non-self-monitoring (NSM) patients is measured in a laboratory after venous blood sampling, which is more invasive, more painful and sometimes problematic, especially in the elderly (7). Although POC devices were originally developed for self-monitoring, POC INR measurement by trained professionals could eliminate the need for venous blood sampling in these NSM patients. However, since most trials regarding POC monitoring have been performed in relatively young and well-functioning patients, capable and willing to perform INR self-monitoring, it is essential to know whether in NSM patients POC monitoring is equally effective and safe as laboratory INR monitoring and whether the quality of anticoagulant control is as good before POC INR measurement can be implemented on a large scale (7). To this day, two randomized controlled trials have evaluated POC INR monitoring by professionals in NSM patients and real-world data to confirm its efficacy and safety in these patients is lacking, which may contribute to the relatively limited implementation of POC INR testing in clinical practice (7-9). Furthermore, in both these trials patients were on warfarin and the impact of POC monitoring by professionals in NSM patients on acenocoumarol or phenprocoumon has not been investigated yet. Therefore, we investigated the efficacy, safety, and the quality of anticoagulant control during POC INR monitoring by trained professionals, as compared with routine laboratory monitoring, in NSM patients of a specialized anticoagulation clinic.

MATERIAL AND METHODS

Study population

POC INR measurement by trained staff of the anticoagulation clinic fully replaced laboratory INR measurement on 29 may 2013 for all NSM patients in the suburbs Capelle aan den IJssel and Krimpen aan den IJssel (Rotterdam, The Netherlands). The study population consisted of two independent cohorts who were each followed up for 1 year. All adult NSM patients were included who received low (therapeutic INR range 2.0-3.5) or high intensity (therapeutic INR range 2.5-4.0) treatment with VKA in these suburbs at 29 May 2012 (laboratory cohort) and at 29 May 2013 (POC cohort). These therapeutic ranges are slightly wider than the internationally advised target ranges (2.0-3.0 and 2.5-3.5 respectively), since the target INR is set higher in the Netherlands (2.5-3.5 and 3.0-4.0) to prevent inadequate anticoagulation (10). Patients were excluded for analysis if they performed self-monitoring during the study period, had less than two valid INR measurements during the 1-year observation period or changed to a deviant therapeutic range during follow-up.

Data collection

We retrieved data from patient records of the Star-Medical Diagnostic Center (Rotterdam, The Netherlands). During each patient visit, nurses of the anticoagulation clinic obtained information regarding complications and changes in medication since the previous visit. INR results, changes in VKA dose, and information obtained during patient visits were registered in the clinics' electronic medical database. In case an appointment for scheduled INR measurement was missed, the clinic contacted the patient, and in case of no contact after several attempts, information regarding current health status was obtained from the patients' general practitioner. Major clinical events (major bleeding, ischemic stroke, hospitalization or death) were either registered during patient visits or reported directly or in retrospect to the clinic by the treating physician. All major adverse events occurring during the course of this study were collected from the medical database six months after follow-up of the POC monitoring cohort ended to minimise the risk of missing any late reported major clinical events. Outcome and severity of all adverse events were registered by specialised physicians of the clinic who were not involved in the study as part of usual medical care. The medical board of the Star-Medical Diagnostic Center approved the use of coded patient data and the study was conducted in accordance with the Helsinki Declaration. The ethics committee of the Erasmus University Medical Center granted a waiver for informed consent due to the observational nature of the study.

Exposure and follow-up

The aim of the study was to compare the safety and quality of anticoagulant control during laboratory and POC INR monitoring in NSM patients. To compare both methods, we included patients who were monitored by the anticoagulation clinic at the start of the laboratory (May 29th 2012) and/or POC (May 29th 2013) observation period. All patients were followed until they ended treatment with VKA, died, or reached the end of the observation period (28 May of the subsequent year). In the analyses regarding clinical outcomes, follow-up ended also when a major clinical event occurred as defined in the section "Outcomes". During both observation periods, monitoring frequency and VKA dosage were based on the patients' INR results, and adjusted if necessary according to the same acenocoumarol and phenprocoumon dosing schedules, which are based on the guidelines of the federation of Dutch Thrombosis Services (10).

INR measurement

For the laboratory INR measurements, 2.7 ml blood was drawn, after venipuncture of the antecubital vein, in to a Becton Dickinson Vacutainer® collection tube (Becton Dickinson, Plymouth, England) containing 0.109M (3.2%) Sodium Citrate. After centrifugation of the venous blood sample for 8 minutes at 2369x*g* at room temperature, plasma INR was determined on the STA-R Evolution automated coagulation system (Diagnostica Stago S.A.S, Asnières sur Seine, France) using the STA Hepato Quick reagent (Roche *Diagnostics*, Mannheim, Germany). POC INR measurements were performed by trained staff of the anticoagulation clinic using the CoaguChek XS Pro system (Roche Diagnostics, Mannheim, Germany). After a finger stick procedure, the first drop of capillary blood was applied to the CoaguChek XS test strip. All different lots of CoaguChek XS test strips and Hepato Quick reagents used during the course of this study were validated and approved for use by the Dutch Reference Laboratory for Anticoagulant Control (RELAC).

Outcomes

To compare the quality of anticoagulant control, we calculated the percentage of time in therapeutic range (TTR) for each patient during the POC and laboratory INR monitoring period. The TTR was calculated using the Rosendaal method (11). If consecutive INR measurements were more than 56 days apart, the monitoring period was censored and not included in the TTR analyses (12). In addition, we made three categories to describe the anticoagulant control of both exposures further: good (TTR >75.0%) moderate (TTR 60-75%) or poor (TTR<60.0%) (2).

To determine the safety of POC INR monitoring, we computed incidence rates of major bleeding, ischemic stroke, hospitalization, and all-cause mortality during laboratory

and POC INR monitoring. Major bleeding was defined as any fatal, or intra-articular or intracranial hemorrhage, or a bleeding that required hospitalization or blood transfusion.
Secondary outcomes were INR testing frequency, percentage of INR results within therapeutic range, and percentage of INR results followed by a significant dose adjustment, defined as any dose adjustment of 10% or more.

Statistical analysis
We used descriptive statistics to summarize baseline characteristics of both exposure groups. The laboratory and POC monitored exposure groups were considered independent samples. We compared TTRs between groups by means of a t-test (in case of a normal distribution) or by Mann-Whitney U test (in case of a skewed distribution). For clinical events, incidence rates and 95% confidence intervals were calculated based on the Poisson distribution. Also, Kaplan-Meier curves were constructed for each exposure group to compare the cumulative incidence of major clinical events and hazard ratios and 95% confidence intervals were estimated by means of a Cox proportional hazard model.

Sensitivity analysis
We performed three sensitivity analyses to verify the validity of our findings. Firstly, a sensitivity analysis was performed on patients who were treated with VKA for at least 6 months at baseline of laboratory and/or POC monitoring, because TTR in the inception period (first 6 months of treatment with VKA) is significantly lower (13). Secondly, we stratified by treatment intensity, as TTR in the low intensity treatment group is higher than in the high intensity treatment group during laboratory monitoring (5). Finally, we stratified by the type of VKA the patients were treated with (acenocoumol or phenprocoumon) because higher TTR levels are reported in patients on phenprocoumon than in patients on acenocoumarol, which may also depend on which method is used for INR monitoring (5). All analyses mentioned above were performed using SPSS version 21.0 (IBM Corp. Armonk, NY, USA).

RESULTS

We included 1973 out of 2006 (98.4%) patients who were registered at baseline of laboratory monitoring and 1959 out of 1999 (98.0%) patients who were registered at baseline of POC monitoring for analysis (see figure 1). Table 1 shows the patient characteristics at baseline of laboratory and POC INR monitoring. Patients were older

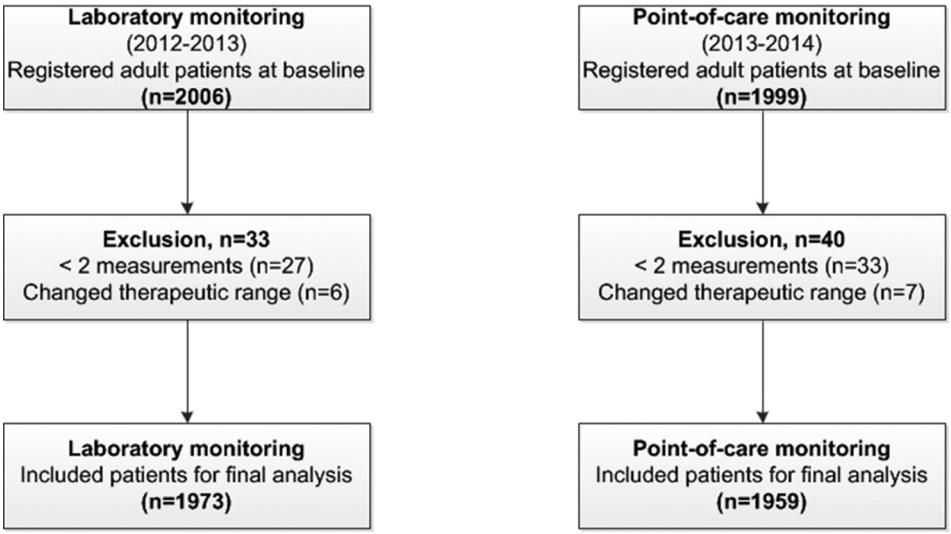


Figure 1. Study flow chart

during POC monitoring than during laboratory monitoring (76.2 vs. 75.3, P=0.02). All other characteristics (sex, INR target range, VKA indication, type of VKA) were similar between exposure groups.

TTR analyses

38,813 INR measurements were performed in 1973 patients during the laboratory-monitoring period, of which 63 INR measurements were excluded from TTR analyses because the maximum interval of 56 days between consecutive measurements was exceeded. During POC monitoring, 41,152 INR measurements were performed in 1959 patients, of which 76 measurements were excluded for TTR analysis for the same reason. TTR levels during laboratory and POC monitoring are shown in table 2.
The median number of evaluated days per patient was 336 (IQ range 307-350) during both exposure periods. Overall, median TTR was lower during POC monitoring (77.9% [95% CI 67.2-87.4]) than during laboratory INR monitoring (81.0%, [95% CI 71.1-90.5]; P<0.001). The proportion of patients achieving a poor TTR (TTR<60.0%) was higher during POC monitoring than during laboratory monitoring, which is shown in table 3.

Clinical events

Incidence rates and hazard ratios of major clinical events during POC and laboratory INR monitoring are shown in table 4. Kaplan-Meier curves for all clinical events were

similar for POC and laboratory monitoring (figure 2, all log-rank P values >0.5). The incidence rate for major bleeding was 17.0 per 1000 patient years (95% CI 11.9-24.3) during POC monitoring and 18.2 per 1000 patient years (95% CI 12.9-25.6) during laboratory monitoring. Incidence rates for ischemic stroke were 6.8 per 1000 patient years (95% CI 3.9-11.9) during POC monitoring and 7.4 per 1000 patient years (95% CI 4.3-12.6) during laboratory monitoring. Adjusted hazard ratios of POC monitoring as compared with laboratory monitoring were 0.93 (95% CI 0.56-1.52) for major bleeding, 0.92 (95% CI 0.42-2.02) for ischemic stroke, 0.94 (95% CI 0.79-1.12) for hospitalization and 1.00 (95% CI 0.78-1.29) for all-cause mortality.

Table 1. Patient characteristics

	Laboratory monitoring		Point-of-care monitoring	
	(n=1973)		(n=1959)	
Mean age (SD), years	75.3	(11.9)	76.2	(11.3)
Male, n (%)	1029	(52.2)	1006	(51.4)
Treatment duration, n (%)				
< 6 months	241	(12.2)	242	(12.4)
≥ 6 months	1732	(87.8)	1717	(87.6)
Therapeutic range, n (%)				
2.0-3.5	1639	(83.1)	13	(85.1)
2.5-4.0	334	(16.9)	292	(14.9)
Treatment indication, n (%)				
Atrial fibrillation	1272	(64.5)	1308	(66.8)
Valve prosthesis	113	(5.7)	105	(5.4)
VTE	238	(12.0)	224	(11.4)
Other	350	(17.8)	322	(16.4)
Type of VKA, n (%)				
Acenocoumarol	1852	(93.9)	1852	(94.5)
Phenprocoumon	121	(6.1)	107	(5.5)

SD=Standard Deviation, VTE=Venous thromboembolism, VKA=Vitamin K antagonist

Secondary outcomes

Percentage of INR results within therapeutic range was lower during POC monitoring than during laboratory monitoring (66.8% vs. 70.1%, P<0.001). INR testing frequency (21.0 vs. 19.7 per patient per year, P<0.001) and percentage of INR followed by a significant adjustment in VKA dose (3.8% vs. 3.3%, P<0.001) were higher during POC monitoring than during laboratory monitoring.

Table 2. Time in therapeutic range during laboratory and point-of-care monitoring

	Laboratory		Point-of-care		
	Patients	**Median TTR [IQR]**	**Patients**	**Median TTR [IQR]**	**P-value ***
Overall	1973	81.0 [71.1-90.5]	1959	77.9 [67.2-87.4]	<0.001
Treatment duration					
< 6 months	241	78.6 [65.8-90.7]	242	76.8 [65.1-86.0]	0.101
≥ 6 months	1732	81.2 [71.8-90.5]	1717	78.0 [67.4-87.5]	<0.001
Therapeutic range					
2.0-3.5	1639	82.0 [72.1-91.2]	1667	78.7 [68.2-88.3]	<0.001
2.5-4.0	334	76.1 [66.6-85.2]	292	73.1 [59.7-81.0]	<0.001
Type of VKA					
Acenocoumarol	1852	80.9 [71.1-90.4]	1852	77.8 [66.9-87.3]	<0.001
Phenprocoumon	121	82.4 [71.0-92.6]	202	81.3 [74.9-92.2]	0.704

* Mann-Whitney U. TTR= Time in therapeutic range in %, VKA= Vitamin K antagonist

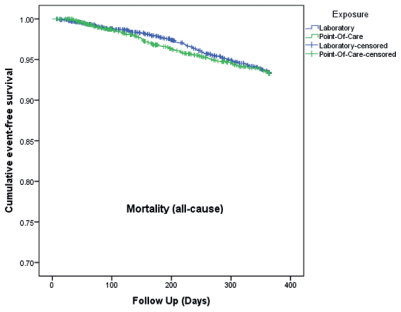
Sensitivity analyses

Sensitivity analyses showed that TTR was lower during POC monitoring as compared with laboratory monitoring, except in phenprocoumon users (see table 2). Also, the proportion of phenprocoumon users achieving a good TTR (>75%) during POC monitoring was higher as compared with laboratory monitoring, while other subgroups showed similar results as the main analyses (i.e. lower TTR during POC monitoring than during laboratory monitoring, see table 3).

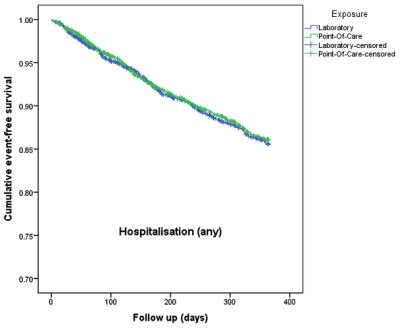
Table 3. Number of patients achieving poor, moderate and good anticoagulation control

	Laboratory		Point-of-care	
	Patients	**Proportion (95% CI)**	**Patients**	**Proportion (95% CI)**
Overall				
Poor	212	10.7 (9.4 - 12.2)	284	14.5 (13.0 - 16.1)
Moderate	452	22.9 (21.1 - 24.8)	536	27.4 (25.4 - 29.4)
Good	1309	66.3 (64.2 - 68.4)	1139	58.1 (55.9 - 60.3)
Treatment duration				
< 6 months				
Poor	47	19.5 (15.0 - 25.0)	48	19.8 (15.2 - 25.2)
Moderate	50	20.7 (16.1 - 26.3)	59	24.4 (19.4 - 30.2)
Good	144	59.8 (53.5 - 65.7)	135	55.8 (49.5 - 61.9)
≥ 6 months				
Poor	165	9.5 (8.2 - 11.0)	236	13.7 (12.2 - 15.5)
Moderate	402	23.2 (21.3 - 25.3)	477	27.8 (25.7 - 30.0)
Good	1165	67.3 (65.0 - 69.4)	1004	58.5 (56.1 - 60.8)
Therapeutic range				
2.0-3.5				
Poor	155	9.5 (8.1 - 11.0)	211	12.7 (11.2 - 14.3)
Moderate	356	21.7 (19.8 - 23.8)	445	26.7 (24.6 - 28.9)
Good	1128	68.8 (66.5 - 71.0)	1011	60.6 (58.3 - 63.0)
2.5-4.0				
Poor	57	17.1 (13.4 - 21.5)	73	25.0 (20.4 - 30.3)
Moderate	96	28.7 (24.2 - 33.8)	91	31.2 (26.1 - 36.7)
Good	181	54.2 (48.8 - 59.5)	128	43.8 (38.3 - 49.6)
Type of VKA				
Acenocoumarol				
Poor	195	10.5 (9.2 - 12.0)	272	14.7 (13.2 - 16.4)
Moderate	432	23.3 (21.5 - 25.3)	521	28.1 (26.1 - 30.2)
Good	1225	66.1 (64.0 - 68.3)	1059	57.2 (54.9 - 59.4)
Phenprocoumon				
Poor	17	14.0 (9.0 - 21.4)	12	11.2 (6.5 - 18.6)
Moderate	20	16.5 (11.0 - 24.2)	15	14.0 (8.7 - 21.9)
Good	84	69.4 (60.7 - 76.9)	80	74.8 (65.8 - 82.0)

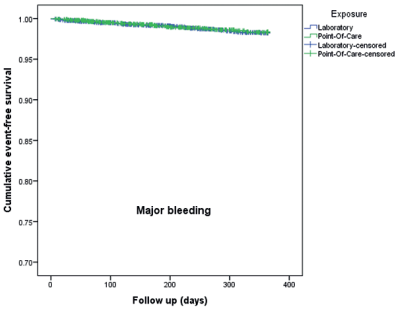
*Poor = TTR<60.0%, †Moderate=TTR 60.0-75.0, ‡Good= TTR >75.0%. CI=Confidence Interval, VKA = Vitamin K antagonist



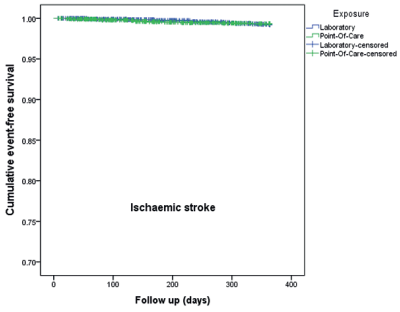
Exposure	Number at risk	Number of events	Log-Rank P value
Laboratory	1973	119	
Point-Of-Care	1959	123	0.81



Exposure	Number at risk	Number of events	Log-Rank P value
Laboratory	1973	261	
Point-Of-Care	1959	252	0.65



Exposure	Number at risk	Number of events	Log-Rank P value
Laboratory	1973	32	
Point-Of-Care	1959	30	0.80



Exposure	Number at risk	Number of events	Log-Rank P value
Laboratory	1973	13	
Point-Of-Care	1959	12	0.84

Figure 2. Kaplan Meier curves of major clinical events

DISCUSSION

The aim of our study was to determine whether point-of-care (POC) INR monitoring by professionals is a safe and effective alternative to laboratory INR monitoring in non-self-monitoring (NSM) patients on vitamin K antagonists (VKA). In such NSM patients, we observed that time in therapeutic range (TTR) was significantly lower during POC monitoring than during laboratory monitoring. Despite the lower TTR, risk estimates indicated that the risk of clinical events such as major bleeding or ischaemic stroke was not increased.

The fact that the TTR was lower during POC monitoring than during laboratory monitoring may be explained by the current dosing algorithms, which are based on laboratory INR results rather than on POC INR results. Although in general agreement between POC and laboratory results has shown to be satisfactory for clinical practice,

Table 4. Hazard ratios of major clinical events during laboratory and point-of-care INR monitoring

	Patients	Events	Person years	Incidence per 1000 py (95% CI)	Hazard ratio (95% CI)	
					Crude	Adjusted *
Mortality (all-cause)						
Laboratory	1973	119	1773	67.1 (56.4 - 79.8)	Reference	Reference
Point-of-care	1959	123	1773	69.4 (58.5 - 82.3)	1.03 (0.80 - 1.33)	1.00 (0.78 - 1.29)
Hospitalization (any)						
Laboratory	1973	261	1658	157.4 (140.8 - 176.0)	Reference	Reference
Point-of-care	1959	252	1669	151.0 (134.8 - 169.2)	0.96 (0.81 - 1.14)	0.94 (0.79 - 1.12)
Major bleeding						
Laboratory	1973	32	1759	18.2 (12.9 - 25.6)	Reference	Reference
Point-of-care	1959	30	1761	17.0 (11.9 - 24.3)	0.94 (0.57 - 1.54)	0.93 (0.56 - 1.52)
Ischemic stroke						
Laboratory	1973	13	1768	7.4 (4.3 - 12.6)	Reference	Reference
Point-of-care	1959	12	1767	6.8 (3.9 - 11.9)	0.92 (0.42 - 2.02)	0.92 (0.42 - 2.02)

* adjusted for age, py= patient-years

we recently have shown that, relative to laboratory results, the Coaguchek XS underestimates INRs ≤ 4.0 and overestimates INRs > 4.0 (14,15). When POC results are interpreted as laboratory results, these discrepancies, especially at sub- and supratherapeutic INR levels, could cause over- or under-dosing of VKA, potentially resulting in a greater degree of INR instability. Such an increase in INR instability was observed in our study, since fewer patients had a good TTR (with the exception of the phenprocoumon users), and more patients required a significant VKA dose adjustment after INR measurement during POC monitoring. Furthermore, we also observed a significantly higher INR testing frequency during POC monitoring, also suggesting less stabile anticoagulation control. The lower TTR and higher testing frequency during POC monitoring might also be explained by the difference in thromboplastin source (tissue-extract in laboratory test versus recombinant in POC test). A previous study has shown method-dependent differences in INR variability during VKA treatment, caused by the differences between thromboplastin reagents in sensitivity to circulating clotting factors II and VII, which fluctuate during VKA treatment (16).

Although TTR was lower during POC monitoring, the most important question remains whether or not there is a negative impact of POC monitoring on patient outcome. The fact that the hazard ratios of clinical events were all around unity indicates that POC monitoring was not associated with an increased risk of adverse events after 1 year of follow-up, although the absolute number of major bleedings and ischemic strokes that

occurred during the study period were relatively low. The similarity in patient outcome may be explained by the fact that, overall, anticoagulant control was still adequate during POC monitoring. According to Dutch guidelines, in long-term patients an average TTR of at least 70% (for a low-intensity treatment group) or 65% (for a high-intensity treatment group) indicates good quality of care (10). In our study, median TTR levels in long-term patients during POC monitoring were well above these thresholds (79.1% in the low-intensity group and 73.2% in the high-intensity group), confirming that, although TTR was lower during POC monitoring, anticoagulant control remained good. However, it should be mentioned that on an individual level, there was an obvious shift in anticoagulant control, with more patients achieving a poor (<60%) or moderate (60-75%) TTR and fewer patients achieving a good TTR (>75%) during POC monitoring as compared with laboratory monitoring. Since poor TTR remains the most important predictor of long-term patient outcome during VKA therapy, methods to optimize anticoagulant control during POC monitoring should be a topic of future research.

Comparison with other studies

To our knowledge, this is the first study using real-world data to investigate the safety and efficacy of POC INR monitoring by professionals at an anticoagulation clinic in NSM patients. Two prior randomized controlled trials in NSM patients on warfarin have reported conflicting results regarding the effect of POC monitoring on TTR, but both studies reported non-inferiority regarding clinical outcome, which is in agreement with our results (8,9). The impact of POC monitoring on TTR has also been studied in clinical trials evaluating INR self-monitoring and management (17-23). A recent meta-analysis of these studies also showed heterogeneous results between trials (24): when after 1 year of follow-up the intervention group was compared with controls receiving standard care, some studies reported higher TTR, while others reported lower TTR (17-23). Potential explanations for these differences could be differences between the trials in INR monitoring frequency, used reference laboratory method, instructions for the POC device or the use of VKA with different half-lives. Our results are in line with those of previous studies that have evaluated self-monitoring and self-management in patients on acenocoumarol and phenprocoumon (21,25). In these trials, POC monitoring was associated with lower TTR in patients on the short-acting VKA acenocoumarol (half-life 2-8h) and higher TTR in patients on the long-acting VKA phenprocoumon (half-life 156-178h) compared to controls receiving laboratory monitoring (21,25,26). Taken together with the results of these trials, our findings suggest that the effect of POC INR monitoring on the TTR may be better when the patient is taking a VKA with a longer half-life (21,25). It would be of interest to investigate whether switching patients to a long-acting VKA improves TTR during POC monitoring.

Strengths and limitations

Our study has three main strengths. Firstly, we had access to a large cohort with complete follow-up and known clinical end points. The large size of the study population enabled us to answer our research question using statistical analyses that had adequate power and robustness, particularly in the case of subgroup analyses. A second strength is that the cohort consisted of an unselected population. This means that our results are generalizable to the real world setting, which consists mainly of elderly patients with atrial fibrillation. Third, due to the large study population, we were able to study clinical endpoints and not only a surrogate for those endpoints (TTR). However, it is important to remark that the duration of follow-up was limited to one year for both monitoring groups and therefore the number of major bleedings and ischemic strokes that occurred during the study period were relatively low.

Our study has three main limitations. First and most importantly, physicians were aware of the implementation of POC monitoring and knew whether the INR result they used to determine dosage was a POC or laboratory result. This could have caused physicians – consciously or subconsciously – to dose differently and monitor patients more intensively during POC monitoring, which may have biased our results. Indeed the higher number of INR measurements during POC monitoring as compared with laboratory monitoring may partly be explained by this bias. However, dosing algorithms and monitoring frequency guidelines were not adjusted during the course of this study and we found no evidence for changes in physicians' adherence to dosing schedules between the two periods of monitoring, suggesting that the impact of this bias on our results was relatively limited. Second, our study was an observational study, meaning that other factors could have influenced the results. Patient characteristics were very similar though in the laboratory and POC monitoring groups and are unlikely to have caused the consistently observed differences in TTR. Finally, laboratory INR measurements were performed using only one type of reagent (Hepato Quick, a tissue-extract thromboplastin) and POC monitoring was performed using only one type of point-of-care device (Coaguchek XS). Therefore our findings cannot be extrapolated directly to settings where a different POC or laboratory method is used. The use of a recombinant thromboplastin for laboratory INR measurements or of another POC device may have a different effect on TTR (5).

In conclusion, we observed that TTR in NSM patients was lower during POC INR monitoring than during laboratory INR monitoring. However, overall TTR remained good and risk estimates indicated similar risks for clinical outcomes (i.e. major bleeding, ischemic stroke, hospitalization and mortality) in the POC and laboratory monitoring groups. Our results indicate that in NSM patients POC INR monitoring by professionals is a safe, effective, and adequate alternative to laboratory INR monitoring.

REFERENCES

1. Holbrook A, Schulman S, Witt DM, Vandvik PO, Fish J, Kovacs MJ, et al. Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e152S-84S.
2. White HD, Gruber M, Feyzi J, Kaatz S, Tse HF, Husted S, et al. Comparison of outcomes among patients randomized to warfarin therapy according to anticoagulant control: results from SPORTIF III and V. Arch Intern Med. 2007;167(3):239-45.
3. Wan Y, Heneghan C, Perera R, Roberts N, Hollowell J, Glasziou P, et al. Anticoagulation control and prediction of adverse events in patients with atrial fibrillation: a systematic review. Circ Cardiovasc Qual Outcomes. 2008;1(2):84-91.
4. Gallagher AM, Setakis E, Plumb JM, Clemens A, van Staa TP. Risks of stroke and mortality associated with suboptimal anticoagulation in atrial fibrillation patients. Thromb Haemost. 2011;106(5):968-77.
5. Federation of Dutch Thrombosis Services. Summary annual medical reports 2013. Available from: http://www.fnt.nl/
6. Leaning KE, Ansell JE. Advances in the Monitoring of Oral Anticoagulation: Point-of-Care Testing, Patient Self-Monitoring, and Patient Self-Management. J Thromb Thrombolysis. 1996;3(4):377-83.
7. Health Quality Ontario. Point-of-Care International Normalized Ratio (INR) Monitoring Devices for Patients on Long-term Oral Anticoagulation Therapy: An Evidence-Based Analysis. Ont Health Technol Assess Ser. 2009;9(12):1-114.
8. Shiach CR, Campbell B, Poller L, Keown M, Chauhan N. Reliability of point-of-care prothrombin time testing in a community clinic: a randomized crossover comparison with hospital laboratory testing. Br J Haematol. 2002;119(2):370-5.
9. Fitzmaurice DA. Oral anticoagulation control: the European perspective. J Thromb Thrombolysis. 2006;21(1):95-100.
10. Federation of Dutch Thrombosis Services. De kunst van het doseren [in Dutch]. 3rd ed.; 2014. Available from http://www.fnt.nl.
11. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost. 1993; 69(3):236-9.
12. Azar AJ, Cannegieter SC, Deckers JW, Briet E, van Bergen PF, Jonker JJ, et al. Optimal intensity of oral anticoagulant therapy after myocardial infarction. J Am Coll Cardiol. 1996;27(6):1349-55.
13. Rose AJ, Hylek EM, Ozonoff A, Ash AS, Reisman JI, Berlowitz DR. Patient characteristics associated with oral anticoagulation control: results of the Veterans AffaiRs Study to Improve Anticoagulation (VARIA). J Thromb Haemost. 2010;8(10):2182-91
14. Christensen TD, Larsen TB. Precision and accuracy of point-of-care testing coagulometers used for self-testing and self-management of oral anticoagulation therapy. J Thromb Haemost. 2012;10(2):251-60.
15. Biedermann JS, Leebeek FW, Buhre PN, de Lathouder S, Barends JP, de Maat MP, et al. Agreement between Coaguchek XS and STA-R Evolution (Hepato Quick) INR results depends on the level of INR. Thromb Res. 2015;136(3):652-7.
16. Solvik UO, Roraas T, Petersen PH, Stavelin A, Monsen G, Sandberg S. The influence of coagulation factors on the in-treatment biological variation of international normalized ratio for patients on warfarin. Scand J Clin Lab Invest. 2014;74(6):470-6
17. Kortke H, Minami K, Breymann T, Seifert D, Baraktaris A, Wagner O, et al. [INR Self-management after mechanical heart valve replacement: ESCAT (Early Self-Controlled Anticoagulation Trial)] INR-Selbstmanagement nach mechanischem Herzklappenersatz: ESCAT (Early Self-Controlled Anticoagulation Trial). Z Kardiol. 2001;90(Suppl 6):118-24.
18. Fitzmaurice DA, Murray ET, McCahon D, Holder R, Raftery JP, Hussain S, et al. Self management of oral anticoagulation: randomised trial. BMJ. 2005;331(7524):1057.

19. Christensen TD, Maegaard M, Sorensen HT, Hjortdal VE, Hasenkam JM. Self-management versus conventional management of oral anticoagulant therapy: A randomized, controlled trial. Eur J Intern Med. 2006;17(4):260-6.
20. Matchar DB, Jacobson A, Dolor R, Edson R, Uyeda L, Phibbs CS, et al. Effect of home testing of international normalized ratio on clinical events. N Engl J Med. 2010; 363(17):1608-20.
21. Menendez-Jandula B, Souto JC, Oliver A, Montserrat I, Quintana M, Gich I, et al. Comparing self-management of oral anticoagulant therapy with clinic management: a randomized trial. Ann Intern Med. 2005;142(1):1-10.
22. Siebenhofer A, Rakovac I, Kleespies C, Piso B, Didjurgeit U, SPOG 60+ Study Group. Self-management of oral anticoagulation reduces major outcomes in the elderly. A randomized controlled trial. Thromb Haemost. 2008;100(6):1089-98.
23. Kaatz S, Elston-Lafata J, Gooldy S. Anticoagulation therapy home and office monitoring evaluation study. J Thromb Thrombolysis 2001;12:111.
24. Heneghan C, Ward A, Perera R, Self-Monitoring Trialist Collaboration, Bankhead C, Fuller A, et al. Self-monitoring of oral anticoagulation: systematic review and meta-analysis of individual patient data. Lancet. 2012;379(9813):322-34.
25. Gadisseur AP, Kaptein AA, Breukink-Engbers WG, van der Meer FJ, Rosendaal FR. Patient self-management of oral anticoagulant care vs. management by specialized anticoagulation clinics: positive effects on quality of life. J Thromb Haemost. 2004;2(4):584-91.
26. Beinema M, Brouwers JR, Schalekamp T, Wilffert B. Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon. Thromb Haemost. 2008;100(6):1052-7.

CHAPTER 5

Monitoring of treatment with vitamin K antagonists: recombinant thromboplastins are more sensitive to factor VII than tissue-extract thromboplastins

Journal of Thrombosis and Haemostasis. 2017;15:500-6

Joseph S. Biedermann[12], Antonius M.H.P. van den Besselaar[3], Moniek P.M. de Maat[1], Frank W.G. Leebeek[1], Marieke J.H.A. Kruip[12]

[1] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[2] Star-Medical Diagnostic Center, Rotterdam, the Netherlands
[3] Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, the Netherlands

ABSTRACT

Background

Differences regarding sensitivity to factor VII (FVII) have been suggested for recombinant human and tissue-extract thromboplastins used for International Normalized Ratio (INR) measurement, but evidence is scarce. Differences in FVII sensitivity are clinically relevant since they can affect INR stability during treatment with vitamin K antagonists (VKA).

Objectives

To determine if commercial thromboplastins react differently to changes in FVII.

Methods

We studied the effect of addition of FVII on the INR in plasma using three tissue-extract (Neoplastin C1+, Hepato Quick, Thromborel S) and three recombinant human (Recombiplastin 2G, Innovin, Coaguchek XS) thromboplastins. Three different concentrations of purified human FVII (0.006, 0.012, and 0.062 µg/mL plasma), or buffer, were added to five certified pooled plasmas of patients using VKA (INR 1.5-3.5). Changes in FVII activity were measured with two bioassays (Neoplastin and Recombiplastin) and relative INR changes were compared between reagents.

Results

After addition of 0.062 µg/mL FVII, FVII activity in the pooled plasmas increased by approximately 20% (Neoplastin) or 32% (Recombiplastin) relative to the activity in pooled normal plasma. All thromboplastins showed dose-dependent INR decreases. The relative INR change in the pooled plasmas significantly differed between the six thromboplastins. No differences were observed amongst recombinant or tissue-extract thromboplastins. Pooled results indicated that the FVII-induced INR change was greater for recombinant than for tissue-extract thromboplastins.

Conclusions

Differences regarding FVII sensitivity exist between various thromboplastins used for VKA monitoring. Recombinant human thromboplastins are more sensitive to FVII than tissue-extract thromboplastins. Therefore, thromboplastin choice may affect FVII-mediated INR stability.

INTRODUCTION

Millions of people worldwide are currently treated with vitamin K antagonists (VKA) for the primary or secondary prevention of arterial and venous thrombosis. Furthermore, VKA will remain the anticoagulant of choice in selected patients, such as those with mechanical heart valves prostheses or with a contraindication to direct oral anticoagulants (DOACS) (1). Treatment with VKA requires monitoring via the international normalized ratio (INR), which is a ratio of the patient's prothrombin time (PT) to the mean PT of healthy individuals, raised to the power of the international sensitivity index (ISI) of the used thromboplastin reagent and instrument (2). During VKA treatment, the INR fluctuates due to several endogenous and exogenous factors such as an intercurrent illness, use of interacting co-medication, and food intake (3). A previous study by Solvik and colleagues has shown that INR variability during VKA treatment can largely be explained by the (residual) activity of clotting factor VII (FVII) and to a lesser extent to plasma levels of clotting factors II (FII) and X (FX) (4). In addition, FVII, the clotting factor with the shortest half-life of approximately 6 hours, fluctuates more than FII and FX during treatment, and the magnitude of its fluctuation also depends on the half-life of the used VKA (5). Pronounced fluctuations have been observed in patients on the short-acting acenocoumarol compared to minimal fluctuations in patients on the long-acting phenprocoumon (5).

The activity of FVII is usually determined by bioassays that use FVII-deficient plasmas and tissue factor (thromboplastin) preparations. It has been shown that the sensitivity of the Factor VII assay system depends on the origin of the thromboplastin; human (placenta-derived) thromboplastin was more sensitive than rabbit thromboplastin (6). Other investigators have reported that recombinant human tissue factor is more sensitive to FVII deficiency than other thromboplastin reagents (7,8). Differences in sensitivity to FVII between recombinant human and tissue-extract thromboplastins used for INR measurement has also been suggested as a source of INR inconsistency, especially during unstable anticoagulation, such as in the initial phase of oral anticoagulant therapy (9,10). If present, these differences in sensitivity to FVII could have a significant effect on the INR and may explain INR discrepancies between methods reported in prior studies (9-12). More importantly, differences in sensitivity to FVII could also directly influence the requirement for VKA dose adjustment, if significant INR discrepancies are present, especially in patients on short-acting VKA (9-11). The conditions of a bioassay specific for FVII are different of those of INR measurement in samples of patients treated with VKA. The purpose of the present study was to determine whether thromboplastins from different sources (e.g. tissue-extract or recombinant human) used in the diagnostic laboratory, react differently to

a fixed increase in FVII. To do so, we measured the INR change in plasma from patients on VKA, induced by spiking the plasma with FVII. We added purified human FVII to patients' plasma and determined the INR change using six different commercially available thromboplastin reagents.

MATERIALS AND METHODS

Five commercially available thromboplastin reagents were used: Dade Innovin, Thromborel S (Siemens Healthcare Diagnostics Products, Marburg, Germany), STA Hepato Quick and Neoplastin C1+ (Diagnostica Stago, Asnières-sur-Seine, France) and Recombiplastin 2G (Instrumentation Laboratory Company, Bedford, Massachusetts). Plasma prothrombin times (PT) were determined in polystyrene tubes using a semi-automatic coagulometer (H. Amelung, Lemgo, Germany) according to Schnitger and Gross (13). Each PT was determined in duplicate on the same coagulometer and the average of the two duplicates were used for further calculations. In addition, PT was also determined using the CoaguChek XS PT Test system, which contains recombinant human thromboplastin (Roche Diagnostics, Mannheim, Germany) (14).
Six certified deep-frozen pooled citrated plasmas were used for the experiments. Of these, one had been prepared by pooling plasmas obtained from 35 healthy individuals. The pooled normal plasma sample was used in the FVII assay to quantify the change in FVII activity after spiking with FVII. Each of the other plasmas had been prepared by pooling approximately 200 plasmas obtained from patients treated with VKA. Individual patients' plasmas were selected according to their INR determined by the Leiden Anticoagulant Clinic and pooled in the following INR intervals: 1.5–2.0, 2.0–2.4, 2.4–2.8, 2.8–3.2, and 3.2–3.6. The pooled plasmas had been stored in 0.5 ml aliquots at -70 °C. All plasmas were thawed for 3 minutes in a water bath at 37 °C before use. Certified PTs and INRs of these plasmas had been determined by three laboratories using the international standards for rabbit thromboplastin (RBT/05) and for recombinant human thromboplastin (rTF/09), according to procedures described previously (15). PTs for the international standards were determined with the manual (tilt-tube) technique in a water bath at 37 °C. Certified INRs were calculated using the ISI values established for the international standards (16,17). The certified INRs were used to calculate instrument-specific ISI and mean normal PT (MNPT) values for the above-mentioned commercial thromboplastins (15). Briefly, logarithms of certified INR were plotted versus logarithms of PTs determined with the commercial thromboplastin and an orthogonal regression line was calculated. ISI and MNPT were calculated from the slope and intercept of the orthogonal regression line (15).

Purified FVII (origin: human blood) was obtained from Haematologic Technologies Inc. (Essex Junction, Vermont). It had a specific activity of 2393 U/mg according to the manufacturer. It was prediluted in buffer (0.15 M NaCl, 20 mM Tris.HCl, pH 7.4, 1% BSA). The diluted FVII solution was stored at 6 □ and used for all experiments between November 2015 and February 2016. The activity was checked regularly and no significant change was observed during this period. The following FVII concentrations were made: 0.16 µg/mL, 0.32 µg/mL, and 1.60 µg/mL. These dilutions of FVII (10 µL) were added to plasma (250 µL), giving a calculated FVII increase of 0.006, 0.012 and 0.062 µg/ml plasma respectively. In control experiments, only buffer was added to plasma. CoaguChek XS measurements were performed by mixing plasma (50 µL) with 16 mM calcium chloride solution (50 µL) in a polystyrene tube, immediately applying 30 µL of the mixture to a test strip that had been inserted in the instrument. PTs were read from the screen and transformed to INR using the instrument-specific ISI and MNPT. FVII activity was determined using Recombiplastin 2G, Neoplastin C1+, pooled normal plasma and FVII-deficient plasma (18). FVII activity in pooled normal plasma was set at 100%. FVII activity and 95% confidence intervals were calculated using a computer program for parallel line assays (CombiStats, version 5.0, European Directorate for the Quality of Medicines and Healthcare, Strasbourg, France).

Statistical analysis

FVII activity in the pooled plasmas of patients on VKA, before and after spiking with the highest concentration of FVII, was compared between Recombiplastin 2G and Neoplastin C1+ using the Wilcoxon signed rank test. Absolute changes in FVII activity in the pooled plasmas were also calculated and compared between reagents using the Wilcoxon signed rank test. INRs obtained for plasmas spiked with FVII were compared with the same plasmas spiked with the same volume of buffer without FVII. The coefficients of variation (imprecision) for each reagent system were calculated by dividing the standard deviation by the means from the duplicate INR results. For further statistical analyses, the mean PT and INR of duplicate measurements were used. Absolute and relative INR changes after addition of different concentrations of FVII were calculated for all pooled plasmas on each reagent system. The Wilcoxon signed rank test and the Friedman test were used to compare the differences in relative INR change between reagent systems. Statistical analyses were performed using SPSS version 22.0 (IBM Corp. Armonk, NY, USA) and figures were constructed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

RESULTS

Certified plasma INR results and FVII activity

Baseline INR values for the certified pooled plasmas are shown in table 1, as well as FVII activities before and after spiking with FVII. Baseline FVII activity levels in these plasmas were significantly higher when measured with Neoplastin C1+ compared to Recombiplastin 2G (P=0.043). The increase in Factor VII activity, as measured using Recombiplastin 2G, was approximately 32% (median change 32.5%, interquartile range 31.5 to 33.4) following addition of 0.062 µg/mL Factor VII. Using Neoplastin C1+, the measured increase in FVII activity was approximately 20% (median change 20.0%, interquartile range 17.0 to 21.0). Considering all five samples of patients on VKA, the absolute increase in FVII activity was significantly greater when measured with Recombiplastin 2G compared to Neoplastin C1+ (P=0.043).

Table 1. Mean INR and Factor VII activity in pooled plasmas before and after addition of 0.062 µg/mL purified FVII

Plasma	Mean INR* (rTF/09)	Mean INR* (RBT/05)	Factor VII activity (95% CI) Recombiplastin 2G		Factor VII activity (95% CI) Neoplastin C1+	
			Before	After	Before	After
12-1	1.51	1.69	40.0 (37.1-43.1)	73.0 (67.5-79.1)	61.3 (57.8-65.1)	79.7 (74.6-85.2)
12-2	2.05	2.27	26.8 (24.3-29.6)	57.7 (53.4-62.4)	43.2 (40.7-45.8)	63.2 (59.7-66.9)
12-3	2.42	2.64	20.1 (18.6-21.7)	51.6 (47.8-55.7)	32.8 (30.8-35.0)	54.9 (51.4-58.6)
12-4	2.77	2.87	17.4 (16.0-18.9)	51.0 (46.5-56.0)	30.2 (28.6-31.9)	44.6 (42.3-47.1)
12-5	3.17	3.28	15.1 (13.9-16.4)	47.4 (44.0-51.1)	25.8 (24.4-27.2)	46.4 (41.7-51.6)

* Mean of the three INR values assigned by the three references laboratories using the manual tilt tube method. INR=International Normalized Ratio, CI=Confidence interval.

INR change after FVII addition using different commercial thromboplastins

Imprecision coefficients of variation for duplicate INR measurement using the coagulometer according to Schnitger and Gross were 0.6% (Hepato Quick), 1.3% (Innovin), 1.7% (Neoplastin C1+), 2.3% (Thromborel S) and 4.5% (Recombiplastin 2G) respectively. The coefficient of variation for the calculated Coaguchek XS INR results was 3.4%. Prothrombin times shortened and INR values decreased with addition of increasing FVII concentrations in all five pooled plasmas of patients on VKA, irrespective of the used thromboplastin reagent (Figure 1). Mean PT and mean INR results of each plasma sample of patients using VKA, after addition of different concentrations of FVII or buffer, are also shown in supplemental table 1 for all reagent systems. Absolute and

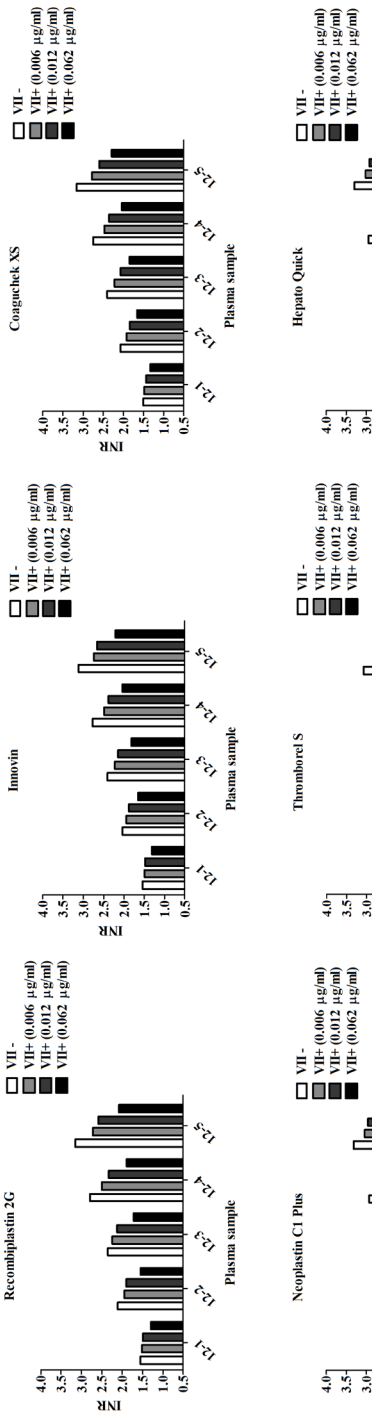


Figure 1. INRs of pooled plasmas after addition of different FVII concentrations or buffer. Each bar indicates the mean INR value of duplicate results from different certified pooled plasmas spiked with FVII or buffer.

relative INR changes after addition of FVII increased with increasing INR level, independent of the administered FVII concentration or the used reagent system (Figure 1). The median proportional INR change and interquartile ranges for the different reagents after spiking with FVII were -13.5% [-26.7 to -7.8] (Recombiplastin 2G), -11.9% [-19.3 to -7.3] (Innovin), -11.9% [-19.9 to -7.4] (Coaguchek XS), -10.3% [-17.3 to -9.0] (Thromborel S), -10.2% [-17.4 to -7.3] (Neoplastin C1+) and -9.1% [-17.0 to -6.0] (Hepato Quick) respectively. Overall, there was a significant difference (P=0.004) between the six thromboplastins regarding the relative change in INR after FVII addition (Figure 2). In contrast, no differences were observed amongst the recombinant human (P=0.085) or tissue-extract (P=0.575) thromboplastins (Figure 2). After combining the data of the three recombinant human and three tissue-extract thromboplastins, the relative INR changes as measured by the recombinant human thromboplastins were significantly higher (P<0.001) than the INR changes as measured using the tissue-extract thromboplastins (Figure 3).

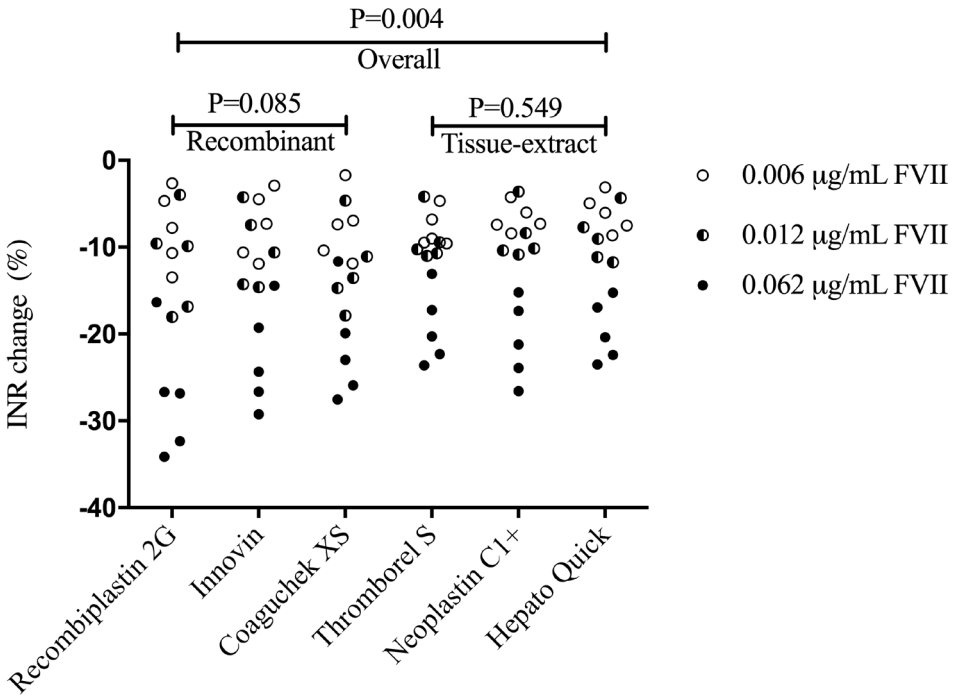


Figure 2. Relative INR changes in pooled plasmas after FVII addition for different thromboplastin reagents. Each dot represents the mean proportional INR change from duplicate results after spiking pooled plasmas from patients on VKA with FVII (n=15). P-value for differences in rank by Friedman test.

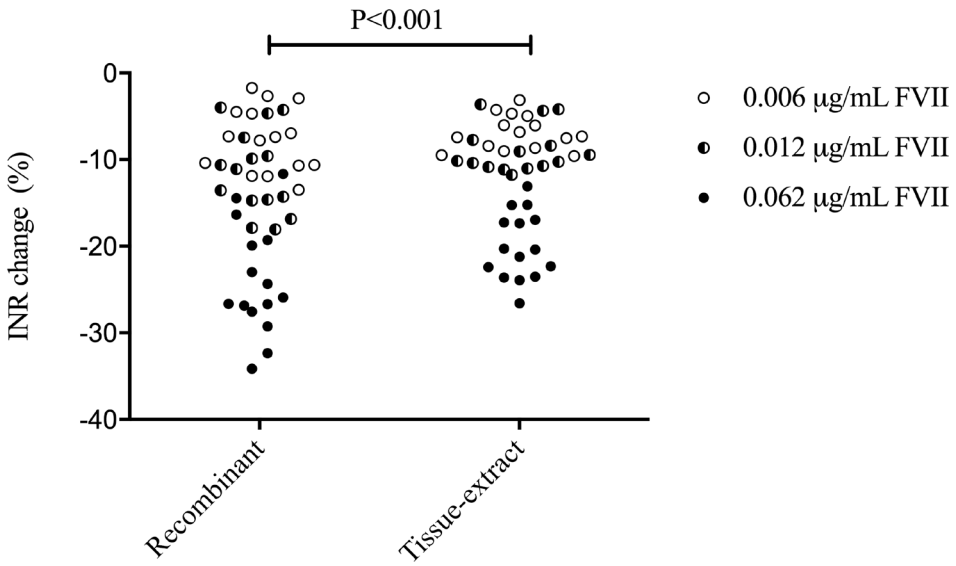


Figure 3. Relative INR changes in pooled plasmas after FVII addition for recombinant versus tissue-extract thromboplastins. Each dot represents the mean proportional INR change from duplicate results after spiking different pooled plasmas with FVII (n=45). P-value for differences in rank by Wilcoxon-signed rank test.

DISCUSSION

In our study, we found that, despite optimal calibration, the relative INR change after FVII addition significantly differed between the six used thromboplastin reagents used for INR measurement of patients on VKA. One of these thromboplastins was used in an electrochemical point-of-care device and the other five in a mechanical coagulometer. Furthermore, the INR change after FVII addition was greater when measured using a recombinant human thromboplastin than when measured using a tissue-extract thromboplastin, suggesting higher sensitivity of recombinant human thromboplastins to FVII. Our findings are in agreement with previous results from Smith and colleagues (19). In their study, recombinant human thromboplastins (Innovin and Recombiplastin) were more sensitive to FVII deficiency than tissue-extract thromboplastins (Thromborel S and Neoplastin C1+) (19). However, as stated by these authors, differences regarding FVII sensitivity, as measured in FVII deficient plasma, may not directly be extrapolated to patients on VKA, since FII and FX levels are also decreased in these patients. As in our study, Smith also added purified human FVII to plasma of patients on VKA, but only reported the change in prothrombin time measured in individual patient plasmas using

synthetic non-commercially available thromboplastins, which complicates the translation to clinical practice. Differences regarding FVII sensitivity between reagents is of clinical importance since it has been shown that patients monitored with the tissue-extract thromboplastin Hepato Quick achieve better anticoagulation control than patients monitored with the recombinant human thromboplastins Innovin and Recombiplastin (20). Furthermore, in a previous study we reported a significant difference in percentage of time in therapeutic range between patients monitored with a laboratory method using Hepato Quick, compared to patients monitored by professionals using the Coaguchek XS point-of-care system (21). The fact that most patients in the Netherlands (19), as well as in the previously mentioned cohort study (21), are treated with the short-acting VKA acenocoumarol may help explain the observed differences in anticoagulation control between patients monitored with reagents of different sources. Circulating FVII levels can fluctuate strongly in these patients and recombinant human thromboplastins seem to be very sensitive to changes in FVII, thus directly affecting measured INR stability (5,22). However, method or reagent-dependent differences in INR stability have also been reported in patients on the longer acting warfarin, emphasizing the importance of our findings beyond patients treated with acenocoumarol (4,23). It has been suggested that the pharmacodynamic effects of warfarin regarding INR variability are more similar to acenocoumarol than to phenprocoumon (24). Also, similar daily FVII fluctuations have been observed in patients on warfarin and on acenocoumarol (25). Clinicians should be aware of these reagent-dependent differences regarding INR stability and include this knowledge in their evaluation of quality of anticoagulation control in their patients. However, it should be noted that reagent-dependent differences in anticoagulation control have not yet been directly translated into higher thrombotic or bleeding complication rates between patients in these studies (21,23). Therefore there is currently no direct requirement for switching to a specific thromboplastin or monitoring method.

Strengths and limitations

We used pooled patients' plasmas for our experiments rather than individual patient plasmas. Pooled patients' plasmas have the advantage that differences between individuals in clotting factor concentrations are averaged. In pooled plasmas, the relationship between INR and clotting factor activity is less variable than the same relationship determined in individual patient plasmas (26). The influence on INR of a given dose of added FVII to pooled plasma is therefore more representative than that of the same dose added to a plasma sample of an individual patient. In an individual patient's plasma, there is greater probability that an INR change is also influenced by variation in other clotting factors.

A limitation of our study is that we used citrated plasma for the Coaguchek XS measurements. The CoaguChek XS system has been designed for non-citrated whole blood rather than for recalcified citrated plasma. Ideally, the sensitivity of the CoaguChek XS system to FVII should be investigated by adding purified FVII to non-citrated whole blood. However, it should be mentioned that the INRs for recalcified pooled plasmas read from the screen of the CoaguChek XS were similar to the certified INR values determined with the International Standard Thromboplastins using the manual tilt tube method. Furthermore, in another study, the overall paired values of recalcified plasma INR values from the CoaguChek XS and a laboratory INR method showed good correlation (27). Good agreement between certified INR and CoaguChek XS values for recalcified plasmas has also been observed in an external quality assessment scheme (28). These findings indicate that recalcified plasma is an acceptable substitute for whole blood for INR measurement on the CoaguChek XS. Another limitation of our study is that we do not know the exact phospholipid composition of the thromboplastins used in our study. It was stated by Smith that the differences in sensitivity to FVII between tissue-derived and recombinant human thromboplastins can be explained by differences in phospholipid composition between reagents, and that the dissimilar sensitivity to FVII may become more apparent when clotting factor deficiencies become more severe (19). However, even if we would know the exact phospholipid composition of the commercial reagents, they would still be different from the compositions used by Smith, thus hampering a direct comparison. Alternatively, the apparent difference in FVII activity between recombinant and tissue-derived thromboplastins (Table 1) might be explained by the influence of other factors present in the plasma of patients on VKA. It has been suggested that rabbit thromboplastin is not sensitive to non-carboxylated proteins induced by VKA (PIVKA), whereas clotting induced by human thromboplastin can be inhibited by PIVKA (29). Inhibition of clotting by PIVKA might reduce the apparent FVII activity measured with recombinant human thromboplastin. Our findings suggest that knowledge of the thromboplastin source (recombinant human or tissue-derived) helps to predict factor FVII sensitivity. Apart from the above-mentioned possible explanations, another explanation for differences in FVII sensitivity between recombinant human and tissue-extract thromboplastins could be that tissue-derived thromboplastins may be contaminated with traces of FVII and FVII(a) (22). It has previously been shown that when traces of FVIIa were added to recombinant human thromboplastins, sensitivity to FVII decreased (22). Furthermore, significant differences regarding FVII sensitivity have been reported between tissue-derived thromboplastins from normal and FVII deficient animals, also suggesting FVII contamination as explanation for differences in FVII sensitivity between thromboplastins (22,30).

In conclusion, we found significant differences in sensitivity to clotting factor VII between commercially available thromboplastins reagents used for INR measurement in patients on VKA. These differences in sensitivity to FVII can explain INR discrepancies between methods reported in prior studies and observations of differences regarding anticoagulation control between patients monitored with thromboplastins from different sources.

REFERENCES

1. Eikelboom JW, Connolly SJ, Brueckmann M, Granger CB, Kappetein AP, Mack MJ, et al. Dabigatran versus warfarin in patients with mechanical heart valves. N Engl J Med. 2013;369(13):1206-14.
2. Poller L. International Normalized Ratios (INR): the first 20 years. J Thromb Haemost. 2004;2(6):849-60.
3. Lee K, Woo HI, Bang OY, On YK, Kim JS, et al. How to use warfarin assays in patient management: analysis of 437 warfarin measurements in a clinical setting. Clin Pharmacokinet. 2015;54(5):517-25.
4. Sølvik UØ, Røraas T, Petersen PH, Stavelin A, Monsen G, Sandberg S. The influence of coagulation factors on the in-treatment biological variation of international normalized ratio for patients on warfarin. Scand J Clin Lab Invest. 2014;74(6):470-6.
5. van Geest-Daalderop JH, Hutten BA, Péquériaux NC, Haas FJ, Levi M, Sturk A. The influence on INRs and coagulation factors of the time span between blood sample collection and intake of phenprocoumon or acenocoumarol: consequences for the assessment of the dose. Thromb Haemost. 2007;98(4):738-46.
6. Brandt JT, Triplett DA, Fair DS. Characterization and comparison of immune-depleted and hereditary Factor-VII-deficient plasmas as substrate plasmas for Factor VII assays. Am J Clin Pathol 1986;85(5):583-9.
7. Bader R, Mannucci PM, Tripodi A, Hirsh J, Keller F, Solleder EM, et al. Multicentric evaluation of a new PT reagent based on recombinant human tissue factor and synthetic phospholipids. Thromb Haemost. 1994;71(3):292-9.
8. Roussi J, Drouet L, Samama M, Sie P. French multicentric evaluation of recombinant tissue factor (Recombiplastin) for determination of prothrombin time. Thromb Haemost 1994;72(5):698-704
9. Remijn JA, Lucas S, Wildeboer B, van Suijlen JD, Adriaansen HJ. Strongly increased international normalized ratio with recombinant Neoplastin R compared with tissue extract Neoplastin Plus in patients initiating oral anticoagulant therapy: implications for anticoagulation dosage. Clin Chem 2008;54(11):1929-31.
10. Remijn JA, Wildeboer B, van Suijlen JD, Adriaansen HJ. Recombinant thromboplastins vs tissue-extract thromboplastins in patients on unstable oral anticoagulant therapy. Clin Chem 2011;57(6):916-7.
11. Biedermann JS, Leebeek FW, Buhre PN, de Lathouder S, Barends JP, de Maat MP, et al. Agreement between Coaguchek XS and STA-R Evolution (Hepato Quick) INR results depends on the level of INR. Thromb Res. 2015;136(3):652-7.
12. Testa S, Morstabilini G, Fattorini A, Galli L, Denti N, D'Angelo A. Discrepant sensitivity of thromboplastin reagents to clotting factor levels explored by the prothrombin time in patients on stable stable oral anticoagulant treatment: impact on the international normalized ratio system. Haematologica. 2002;87(12):1265-73.
13. Schnitger H, Gross R. A universal apparatus for automatic recording of the clotting time. Klin Wochenschr 1954;32(41-42):1011-2.
14. Leichsenring I, Plesch W, Unkrig V, Kitchen S, Kitchen DP, Maclean R, et al. Multicentre ISI assignment and calibration of the INR measuring range of a new point-of-care system designed for home monitoring of oral anticoagulation therapy. Thromb Haemost. 2007;97(5):856-61.
15. Van den Besselaar AM, Barrowcliffe TW, Houbouyan-Reveillard LL, Jespersen J, Johnston M, Poller L, et al. Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination. J Thromb Haemost 2004;2(11):1946-53.
16. Chantarangkul V, van den Besselaar AM, Witteveen E, Tripodi A. International collaborative study for the calibration of a proposed international standard for thromboplastin, rabbit, plain. J Thromb Haemost 2006;4(6):1339-45.

17. Tripodi A, Chantarangkul V, van den Besselaar AM, Witteveen E, Hubbard AR, for the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the ISTH. International collaborative study for the calibration of a proposed international standard for thromboplastin, human, plain. J Thromb Haemost 2010;8(9):2066-8.
18. Mariani G, Liberti G, D'Angelo T, Lo Coco L. Factor VII activity and antigen. In: Jespersen J, Bertina RM, Haverkate F, editors. Laboratory techniques in thrombosis – A manual. Dordrecht: Kluwer Academic Publishers; 1999. p. 99-106.
19. Smith SA, Comp PC, Morrissey JH. Phospholipid composition controls thromboplastin sensitivity to individual clotting factors. J Thromb Haemost 2006;4(4):820-7.
20. Federation of Dutch Thrombosis Services. Summary annual medical reports 2014. http://www.fnt.nl/ (Accessed April 2016).
21. Biedermann JS, van Rein N, van den Besselaar AM, Buhre PN, de Maat MP, van der Meer FJ, et al. Impact of point-of-care INR monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: a cohort study. J Thromb Haemost 2016;14(4):695-703.
22. Smith SA, Comp PC, Morissey JH. Traces of factor VIIa modulate thromboplastin sensitivity to factors V, VII, X and prothrombin. J Thromb Haemost. 2006;4(7):1553-8.
23. Onundarson PT, Francis CW, Indridason OS, Arnar DO, Bjornsson ES, Magnusson MK, Juliusson SJ, Jensdottir HM, Vidarsson B, Gunnarsson PS, Lund SH, Gudmundsdottir BR. Fiix-prothrombin time versus standard prothrombin time for monitoring of warfarin anticoagulation: a single centre, double-blind, randomised, non-inferiority trial. Lancet Haematol. 2015;2(6):e231-40.
24. van den Besselaar AM, Biedermann JS, van der meer FJ, Adriaansen HJ, Leebeek FW, Kruip MJ. Control of Anticoagulation with vitamin K antagonists: overestimation of median time in therapeutic range when assessed by linear interpolation. Thromb Haemost 2016;116(4):679-86.
25. Barcellona D, Vannini ML, Fenu L, Balestrieri C, Marongiu F. Warfarin or acenocoumarol: which is better in the management of oral anticoagulants. Thromb Haemost 1998;80(6):899-902.
26. Bertina RM. The relationship between the international normalized ratio and the coumarin-induced coagulation defect. In: Van den Besselaar AMHP, Gralnick HR, Lewis SM, eds. Thromboplastin Calibration and Oral Anticoagulant Control. Boston: Martinus Nijhoff Publishers, 1984:223-34.
27. Kim SJ, Lee EY, Park R, Kim J, Song J. Comparison of prothrombin time derived from CoaguChek XS and Laboratory Test according to fibrinogen level. J Clin Lab Anal 2015;29(1):28-31.
28. Tientadakul P, Chuntarut A. Establishing an external quality assessment scheme for point-of-care international normalized ratio in Thailand. Int J Lab Hematol 2015;37(4):509-14.
29. Denson KW, Reed SV, Haddon ME, Woodhams B, Brucato C, Ruiz J. Comparative studies of rabbit and human recombinant tissue factor reagents. Thromb Res 1999;94(4):255-61.
30. Spurling NW, Savory J. The influence of residual factor VII on the sensitivity of brain thromboplastin. Thromb Haemost 1978;39(3):592-9.

Supplemental Table 1. Prothrombin times and INR results after addition of FVII or buffer using six commercial thromboplastins

	Recombiplastin		Innovin		Coaguchek XS		Thromborel S		Neoplastin C1+		Hepato Quick	
	Mean PT	INR	Mean PT	INR	Mean PT	INR	Mean PT	INR	Mean PT	INR	Mean PT	INR
12-0												
FVII –	10.20	0.98	9.63	0.99	12.90	1.00	13.40	0.98	12.55	1.03	27.10	1.03
FVII + (0.006 µg/mL)	10.65	1.02	9.45	0.97	12.84	0.99	12.30	0.90	12.33	1.01	26.95	1.02
FVII + (0.012 µg/mL)	10.40	0.99	9.55	0.98	12.80	0.99	12.65	0.92	12.23	1.00	26.20	1.00
FVII + (0.062 µg/mL)	9.85	0.95	8.90	0.92	12.25	0.95	12.00	0.87	11.60	0.93	24.85	0.95
12-1												
FVII –	16.95	1.55	16.03	1.54	20.05	1.50	20.65	1.52	18.55	1.65	47.87	1.65
FVII + (0.006 µg/mL)	16.45	1.51	15.50	1.49	19.90	1.48	19.70	1.45	17.90	1.58	46.10	1.60
FVII + (0.012 µg/mL)	16.20	1.49	15.25	1.47	19.05	1.43	19.80	1.46	18.00	1.59	45.40	1.58
FVII + (0.062 µg/mL)	13.90	1.30	13.40	1.32	17.55	1.33	18.00	1.32	16.20	1.40	39.30	1.40
12-2												
FVII –	23.85	2.11	22.13	2.04	28.25	2.07	29.10	2.16	23.85	2.25	69.43	2.26
FVII + (0.006 µg/mL)	21.80	1.94	21.00	1.95	25.90	1.93	27.15	2.01	22.67	2.11	65.35	2.15
FVII + (0.012 µg/mL)	21.25	1.90	20.25	1.89	24.90	1.84	25.95	1.92	22.20	2.06	63.10	2.09
FVII + (0.062 µg/mL)	16.85	1.54	17.30	1.64	22.25	1.66	23.30	1.72	20.40	1.86	55.65	1.88
12-3												
FVII –	26.90	2.35	26.73	2.40	33.1	2.40	32.75	2.44	26.75	2.59	82.07	2.60
FVII + (0.006 µg/mL)	25.50	2.24	24.50	2.23	30.0	2.22	29.70	2.20	25.13	2.40	76.20	2.44
FVII + (0.012 µg/mL)	24.05	2.12	23.50	2.15	28.3	2.07	29.30	2.17	24.50	2.32	73.30	2.37
FVII + (0.062 µg/mL)	19.05	1.72	19.40	1.82	25.0	1.85	27.20	2.02	22.00	2.04	62.55	2.07
12-4												
FVII –	32.65	2.80	31.63	2.78	38.45	2.76	37.25	2.78	29.65	2.93	95.03	2.94
FVII + (0.006 µg/mL)	28.80	2.50	27.80	2.48	33.70	2.47	33.75	2.51	27.83	2.71	86.60	2.72
FVII + (0.012 µg/mL)	26.60	2.33	26.50	2.38	32.40	2.35	33.80	2.52	27.10	2.63	82.55	2.61
FVII + (0.062 µg/mL)	21.15	1.89	22.15	2.04	27.85	2.04	28.60	2.12	23.70	2.23	70.25	2.28
12-5												
FVII –	37.30	3.15	36.10	3.12	44.55	3.16	41.25	3.08	32.85	3.32	109.10	3.30
FVII + (0.006 µg/mL)	31.75	2.73	31.20	2.75	38.30	2.79	37.60	2.80	30.57	3.04	97.95	3.02
FVII + (0.012 µg/mL)	29.90	2.58	30.10	2.66	36.05	2.60	37.10	2.77	29.90	2.96	94.00	2.92
FVII + (0.062 µg/mL)	23.45	2.08	24.25	2.21	31.50	2.29	32.20	2.39	25.50	2.44	79.30	2.53

CHAPTER 6

Major bleeding risks of different low-molecular-weight-heparin agents: a cohort study in 12 934 patients treated for acute venous thrombosis

Journal of thrombosis and haemostasis. 2017;15:1386-91

Nienke. van Rein[1,2], J.S. Biedermann[3,4], Felix J.M. van der Meer[1], Suzanne C. Cannegieter[5], N. Wiersma[6], Helga.W. Vermaas[7], Pieter.H. Reitsma[1,2], Marieke.J.H.A. Kruip[3,4], Willem.M. Lijfering[1,2,5]

[1] Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, the Netherlands
[2] Eindhoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands
[3] Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands
[4] Star-Medical Diagnostic Center, Rotterdam, The Netherlands
[5] Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands
[6] Anticoagulation Clinic Utrecht, Utrecht, the Netherlands
[7]Anticoagulation Clinic the Hague, The Hague, the Netherlands

ABSTRACT

Background
Low-molecular-weight-heparins (LMWHs) are considered members of a class of drugs with similar anticoagulant properties. However, pharmacodynamics and pharmacokinetics between LMWHs differ, which may result in different bleeding risks. As these agents are used by many patients, small differences may lead to a large effect on numbers of major bleeding events.

Objectives
To determine major bleeding risks for different LMWH agents and dosing schedules.

Methods
Cohort with acute venous thrombosis patients from four anticoagulation clinics who used a LMWH and a vitamin K antagonist. Patients were followed until they ceased LMWH treatment or until major bleeding. Exposures were classified according to different types of LMWHs and for b.i.d. and o.d. use. Cumulative incidences for major bleeding per 1000 patients and risk ratios were calculated and adjusted for study center.

Results
The study comprised of 12934 patients with a mean age of 59 years; 6218 (48%) men. The cumulative incidence for major bleeding was 2.5 per 1000 patients (95% confidence interval [CI] 1.7-3.5). Enoxaparin b.i.d. or o.d. was associated with a relative bleeding risk of 1.7 (95% CI, 0.2-17.5) compared with nadroparin o.d. In addition, a nadroparin b.i.d. dosing schedule was associated with a 2.0-fold increased major bleeding risk (95% CI, 0.8-5.1) as compared with a nadroparin o.d. dosing schedule.

Conclusions
Absolute major bleeding rates were low for all LMWH agents and dosing schedules in a large unselected cohort. Nevertheless, twice-daily dosing with nadroparin appeared to be associated with an increased major bleeding risk as compared with once-daily dosing, as also suggested in a meta-analysis of controlled clinical trials.

BACKGROUND

Low-molecular-weight-heparins (LMWHs) are widely used for prevention and treatment of venous thrombosis (the composite of deep vein thrombosis and pulmonary embolism) (1). The LMWHs currently on the market are considered members of a class of drugs with similar anticoagulant properties. However, pharmacodynamics and pharmacokinetics between LMWHs differ. For example, the half-life of the anti-Xa activity is 4.3 h for enoxaparin as compared with 2.4 h for dalteparin (2). Such differences in duration of anticoagulant effect could result in different bleeding risks between LMWH agents. For the acute treatment of venous thrombosis, a head-to-head trial was conducted that compared two different LMWHs, i.e. (dalteparin omni die [o.d., i.e. once daily] vs. tinzaparin o.d.). In this randomized study, with 505 patients, the relative bleeding risk of dalteparin versus tinzaparin was 0.40 (95% confidence interval [CI], 0.08 to 2.07) but as the confidence intervals included unity the authors concluded that the bleeding rate was similar for both LMWHs (3). To prevent venous thrombosis after a spinal cord injury with an LMWH, head-to-head trials were performed to compare dalteparin with enoxaparin, which showed that overall bleeding rates were similar (4,5), whereas others showed that enoxaparin had a more favorable risk profile with respect to major bleeding as compared with dalteparin (6). It is currently unclear whether one LMWH should be preferred over the other in the treatment of venous thrombosis, even though small differences in major bleeding rates may lead to a large reduction in major bleeding events, because these agents are used by many patients on an annual basis.

We therefore set out to perform a cohort study in 12 934 patients with acute venous thrombosis who were treated with LMWH (and concurrently received a vitamin K antagonist [VKA]) to determine the major bleeding risk for several LMWH agents and dosage schedules.

METHODS

Study population and data collection

All patients over 18 years of age, with a new onset of venous thrombosis between 2006 and 2013, who received initial treatment with an LMWH (nadroparin o.d., nadroparin bis in die [b.i.d., i.e. twice daily], tinzaparin o.d., enoxaparin o.d. or b.i.d., dalteparin o.d.) and a VKA and were treated at one of the four participating anticoagulation clinics in the Netherlands (Leiden, Rotterdam, The Hague and Utrecht) were included. Enoxaparin o.d. and b.i.d. treatments were combined because numbers were too small.

New onset was defined as an acute diagnosis of a first or recurrent venous thrombosis. Diagnoses were made at hospitals and were based on the international diagnosis criteria, after which patients received therapeutic dosages of LMWHs for at least 5 days (7). At the anticoagulation clinics, patients with acute venous thrombosis are registered. There, the international normalized ratio (INR) is measured at least every 3 days when VKA and LMWH treatment are combined. LMWH treatment is ceased after at least 5 days of treatment and when two consecutive INRs are in the target range (7). At each appointment blood is drawn to measure the INR and a standardized short questionnaire is administered (and electronically stored) by a nurse to list any changes in co-medication or onset of new diseases and to enquire if patients experienced bleeding events or have any surgical procedures planned (8). If patients missed their appointment, they are contacted by nurses of the anticoagulation clinic and a new appointment is made at short notice.

Patient characteristics and information regarding major bleeding events were derived from the computerized medical records of the anticoagulation clinics. Data included age, sex, indication for VKA treatment, type of LMWH, duration of LMWH exposure, concomitant drug use and date of major bleeding events. Patients were considered exposed to an LMWH from the subscription date at the anticoagulant clinic until ceasing treatment with the LMWH plus an additional two days wash-out period, or until a patient died, changed anticoagulation clinic or experienced a major bleeding, whichever occurred first. Of note, in this study it was not planned that the patient records were used before the data collection took place, which could have resulted in less exact data. However, the data needed for this study were necessary for patient care and systematically collected during treatment as part of patient care.

Outcome

The outcome of our study was non-traumatic major bleeding. Bleedings were considered major if they required blood transfusion (mainly red blood cells), were symptomatic in a critical area or organ, or led to death (9). In addition, bleedings for which patients were hospitalized were also considered major bleedings. Bleedings were notified during the standardized short histories taken by specially trained nurses during every visit to the anticoagulation clinic. If patients mentioned any bleeding event or hospitalization related to a bleeding, the information was noted in the electronically stored patient system and trained physicians from the anticoagulation clinic who were not involved in the study obtained information from the hospital, patient or general practitioner to classify the bleeding event as minor or major.

Statistical analysis

Patients were considered exposed to an LMWH from the subscription date at the anticoagulant clinic until date of ceasing treatment with LMWHs plus an additional 2 days washout period. Risks (cumulative incidences) and risk ratios were calculated and adjusted for study center by Mantel–Haenszel methods (10). Nadroparin o.d. was *a priori* chosen as the reference category in these analyses, as this was the most frequently used type of LMWH treatment. We adjusted only for study center as the choice of an LMWH agent is determined hospital-wide and therefore not related to patient characteristics. Furthermore, the choice of the vitamin K antagonist is strongly associated with the anticoagulation clinic, on which basis we assume that no other confounding factors than the anticoagulation clinic are present. A sensitivity analysis was performed where results were restricted to first venous thrombotic events because patients with a recurrent venous thrombotic event have different patient characteristics that also relate to bleeding risk (11). As a second sensitivity analysis, we computed incidence rates per 100 person-years to confirm that incidence rates would yield a similar pattern of risk estimates as cumulative incidences. Follow-up started from registration at the anticoagulation clinic until LMWH treatment was ceased, or the patient moved to a city that was not covered by the participating anticoagulation clinics, died or experienced a major bleeding, whichever occurred first. The maximum follow-up was 90 days.

All analyses were performed with R version 2.15.2 (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

RESULTS

The study cohort comprised 12 934 patients who experienced an acute venous thrombotic event (Figure 1 and Table 1). The mean age at baseline was 59 years (standard deviation, 17 years) and 6218 (48%) patients were male. Most patients were treated at the anticoagulation clinic in Rotterdam (3883 patients; 30%). A deep vein thrombosis (DVT) was the indication of treatment for 8058 patients (62%) and 4889 patients (38%) experienced a pulmonary embolism (PE). In 11 237 (87%) patients the thrombotic episode concerned a first event; 6735 of these experienced a DVT. The remaining 1697 (13%) patients had a recurrent thrombotic episode, of whom 1326 experienced a DVT. The most frequently used LMWHs were nadroparin o.d. (5317 patients) and tinzaparin o.d. (3338 patients).

Table 1. Patient characteristics

	Nadroparin o.d.	Nadroparin b.i.d.	Tinzaparin o.d.	Enoxaparin	Dalteparin o.d.
General characteristics					
Patients	5317	2076	3338	264	1939
Male sex, n (%)	2657 (50)	904 (44)	1638 (49)	125 (47)	894 (46)
Age, years, mean (SD)	58 (17)	61 (18)	59 (16)	57 (17)	57 (17)
Indication VKA treatment					
Deep vein thrombosis, n (%)	3402 (64)	1302 (63)	2042 (61)	137 (52)	1175 (61)
First, n (%)	2735 (80)	1093 (84)	1735 (85)	115 (84)	1057 (90)
Recurrent, n (%)	667 (20)	212 (16)	307 (15)	22 (16)	118 (10)
Pulmonary embolism, n (%)	1924 (36)	776 (37)	1298 (39)	127 (48)	764 (39)
First, n (%)	1695 (88)	731 (93)	1220 (94)	122 (96)	761 (100)
Recurrent, n (%)	229 (12)	59 (8)	78 (6)	5 (4)	3 (0)
Vitamin K antagonist					
Acenocoumarol, n (%)	1399 (26)	1059 (51)	2204 (66)	223 (84)	1861 (96)
Phenprocoumon, n (%)	3918 (74)	1017 (49)	1134 (34)	41 (16)	78 (4)
Co-medication					
Antihypertensive(s), n (%)	1137 (21)	373 (18)	511 (15)	38 (14)	54 (3)
Antidiabetic(s), n (%)	376 (7)	179 (9)	248 (7)	20 (8)	151 (8)
NSAID(s), n (%)	384 (7)	135 (7)	248 (7)	21 (8)	187 (10)
Anti-platelet drug(s), n (%)	152 (3)	100 (5)	138 (4)	13 (5)	112 (6)
Anticoagulation clinic					
The Hague, n (%)	2409 (45)	611 (29)	587 (18)	18 (7)	20 (1)
Leiden, n (%)	1873 (35)	544 (26)	729 (22)	62 (23)	30 (2)
Rotterdam, n (%)	347 (7)	561 (27)	1079 (32)	89 (34)	1807 (93)
Utrecht, n (%)	688 (13)	360 (17)	943 (28)	95 (36)	82 (4)

o.d., once daily; b.i.d., twice daily; NSAID, non-steroidal anti-inflammatory drugs; SD, standard deviation.

The median duration of treatment with LMWH (i.e. follow-up) was 5 days (interquartile range, 2 to 9 days, Table 2). In total, 32 of the 12 934 patients (corresponding cumulative incidence 2.47 per 1000 patients; 95% CI 1.74 to 3.49) experienced a major bleeding event during combined VKA and LMWH treatment. The cumulative incidence (per 1000 patients) of major bleeding during combined treatment was 2.07 (95% CI, 1.11 to 3.75) in nadroparin o.d. users, 3.37 (95% CI, 1.48 to 7.10) in nadroparin b.i.d. users, 2.70 (95% CI, 1.33 to 5.20) in tinzaparin o.d. users, 3.79 (95% CI, 0.00 to 23.34) in enoxaparin o.d. or b.i.d. users and 2.06 (95% CI, 0.60 to 5.50) in dalteparin o.d. users (Table 2). The sensitivity analysis with incidence rates showed a similar pattern of risk estimates as

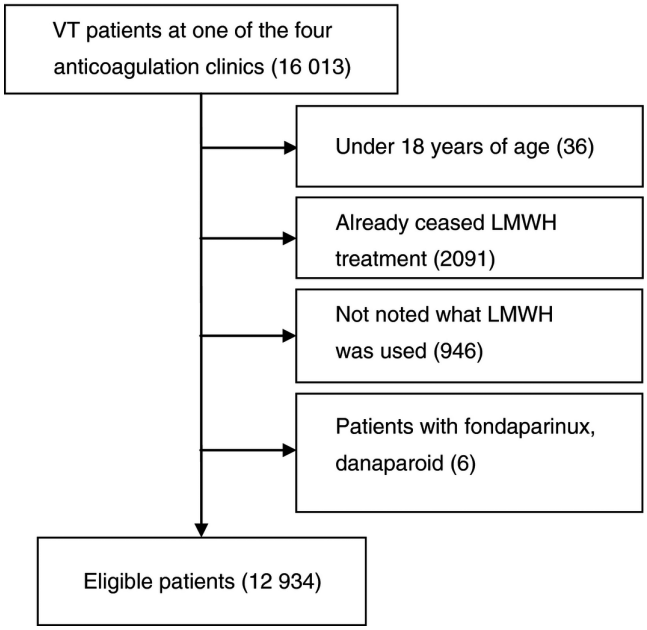


Figure 1. Flow chart of patients. VT, venous thrombosis; LMWH, low-molecular-weight heparin.

analysis with cumulative incidences, although incidence rates of major bleeding were high (7.3 to 16.7 per 100 person-years) (Supplementary Table 1). Risk ratios for the different LMWH agents were between 1.00 and 1.83 using nadroparin o.d. as reference LMWH and increased after adjustment for study center (Table 2). The relative risk estimate for nadroparin b.i.d. was 1.98 (95% CI, 0.76 to 5.14) as compared with nadroparin o.d. treatment. Patients with a first venous thrombotic event had similar cumulative incidences of major bleeding as patients with recurrent events.

DISCUSSION

In this study, the absolute risk of major bleeding was low among patients who were registered at the anticoagulation clinic with an acute venous thrombotic event (between 2.0 to 3.5 per 1000 patients during combined treatment with VKA and LMWH), which is similar to cumulative incidences reported in clinical trials (12). These figures indicate that the combination of a VKA and LMWH for a short period is relatively safe for these patients. Nevertheless, incidence rates of major bleeding were high (7.3 to 16.7 per 100 person-years), indicating that the combination of VKAs and LMWHs should be used

for as short a time as possible. The relative risk estimates for major bleeding events were highest in patients treated with nadroparin b.i.d. and enoxaparin o.d. or b.i.d.. These results should nevertheless be interpreted with caution because numbers were small and confidence intervals showed that a similar risk of bleeding events for these LMWHs as compared with the other LMWHs cannot be ruled out. Given the small numbers, enoxaparin o.d. or b.i.d. treatment was associated with a higher bleeding risk than nadroparin o.d., tinzaparin o.d. and dalteparin o.d. treatment. These results are similar to what was found in a meta-analysis (12), where all LMWHs gave lower bleeding risk as compared with unfractionated heparin (UFH) (nadroparin odds ratio [OR], 0.41; 95% CI, 0.14-1.17; tinzaparin OR, 0.30; 95% CI, 0.12-0.73; dalteparin OR, 0.15; 95% CI, 0.02-1.44), whereas enoxaparin gave risk estimates around unity (OR, 1.14; 95% CI, 0.50-2.16). Our results also indicate that nadroparin o.d. users were at lower risk for bleeding complications than nadroparin b.i.d. users. Although we cannot say with certainty that nadroparin b.i.d. dosing is more harmful as compared with o.d. dosing, these results are in line with a Cochrane review from previous trials in acute venous thrombosis patients. This review suggested that b.i.d. treatment with an LMWH results in higher bleeding rates as compared with o.d. treatment (relative risk, 1.29;

Table 2. Bleeding events associated with LMWH treatment

	Patients	Events	Median LMWH treatment period (IQR), days	Cumulative incidence per 1000	Risk ratio (95% CI)	Risk ratio* (95% CI)
All venous thrombotic events						
Nadroparin o.d.	5317	11	6 (3 to 10)	2.07 (1.11-3.75)	reference	reference
Nadroparin b.i.d.	2076	7	5 (2 to 10)	3.37 (1.48-7.10)	1.63 (0.63-4.20)	1.98 (0.76-5.14)
Tinzaparin o.d.	3338	9	5 (2 to 8)	2.70 (1.33-5.20)	1.30 (0.54-3.14)	1.24 (0.46-3.58)
Enoxaparin	264	1	5 (2 to 8)	3.79 (0.00-23.34)	1.83 (0.24-14.13)	1.74 (0.17-17.46)
Dalteparin o.d.	1939	4	4 (2 to 7)	2.06 (0.60-5.50)	1.00 (0.32-3.13)	4.19 (0.47-37.00)
First venous thrombotic event						
Nadroparin o.d.	4425	9	6 (3 to 10)	2.03 (1.01-3.93)	reference	reference
Nadroparin b.i.d.	1805	7	5 (2 to 9)	3.88 (1.70-8.16)	1.91 (0.71-5.11)	2.32 (0.85-6.31)
Tinzaparin o.d.	2953	8	5 (2 to 8)	2.71 (1.27-5.44)	1.33 (0.51-3.45)	2.30 (0.92-5.78)
Enoxaparin	273	1	5 (2 to 8)	3.66 (0.00-22.58)	1.80 (0.23-14.16)	1.98 (0.19-21.09)
Dalteparin o.d.	1819	3	4 (2 to 7)	1.65 (0.32-5.08)	0.81 (0.22-2.99)	3.97 (0.42-37.59)

* Adjusted for study center and academic hospital by Mantel–Haenszel method. LMWH, low-molecular-weight heparin; IQR, interquartile
range; CI, confidence interval; o.d., once daily; b.i.d., twice daily

95% CI, 0.79-2.50) (13). In addition, another study showed that o.d. dosing of enoxaparin was associated with fewer major bleeding events as compared with b.i.d. dosing (14). Furthermore, one trial showed that nadroparin b.i.d. gave higher rates of bleeding complications as compared with o.d. (relative risk, 1.64; 95% CI, 0.74-3.57) (15). When combining results from our study and the latter study in a *post hoc* meta-analysis with a random effects model, the OR indicates a 1.77 increased risk (95% CI, 0.97-3.23) for patients using b.i.d. nadroparin as compared with o.d. nadroparin.

Some methodological aspects of our study need comment. First, this study evaluated bleeding risks in a large population of unselected venous thrombosis patients from four anticoagulation clinics, which makes our results generalizable to the community. However, as a limitation, patients were included after registration at the anticoagulation clinic, which is usually a couple of days after the diagnosis of a venous thrombotic event at the hospital. During these few days, we could have missed the bleeding events. If so, this would have influenced the absolute bleeding rates found in our study. Therefore, our results are only applicable to patients discharged from hospital. A second limitation is that few bleeding events occurred, which resulted in statically non-significant differences and prevented us from performing several subgroup analyses in patients who are potentially at high risk of major bleeding. The small numbers also hamper the robustness of our results and may have inflated the risk estimate of bleeding events in the enoxaparin o.d. or b.i.d. group. In addition, we were not able to stratify enoxaparin treatment by o.d. and b.i.d. use because of small numbers and we had no patients who used a dalteparin b.i.d. regimen. Another limitation is that we were only able to adjust for study center. However, the choice of LMWH is not based on patient characteristics, but based on the preference of the hospital where the patient presents him or herself for a specific type of LMWH. Therefore, we assume that patient characteristics are not associated with the type of LMWH prescribed and therefore consider it unlikely that residual confounding has influenced our results. Still, we cannot rule out that doctors may have opted, for example, to treat patient with low renal function with twice-daily LMWH instead of once-daily LMWH, which could have led to residual confounding in our study. Therefore, results might have been more precise if our study had been set up to specifically answer the question being asked in this study, as the dosing schedule of LMWH would have been more appropriately documented as well as other details.

In conclusion, the absolute risk for major bleeding complications during treatment with LMWH and VKA in patients with an acute venous thrombosis who were treated at an anticoagulation clinic was low, with an approximate risk of 2.5 per 1000 patients. These low risks prevented us from concluding whether one LMWH should be preferred over the other. Furthermore, nadroparin b.i.d. appeared to be associated with an increased risk of major bleeding as compared with nadroparin o.d., which is in accordance with the literature.

REFERENCES

1. Kearon C, Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ, et al. Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012; 141(2 Suppl):e419S-94S.
2. White RH, Ginsberg JS. Low-molecular-weight heparins: are they all the same? Br J Haematol. 2003;121(1):12-20.
3. Wells PS, Anderson DR, Rodger MA, Forgie MA, Florack P, Touchie D, et al. A randomized trial comparing 2 low-molecular-weight heparins for the outpatient treatment of deep vein thrombosis and pulmonary embolism. Arch Intern Med. 2005; 165(7):733-8.
4. Slavik RS, Chan E, Gorman SK, de Lemos J, Chittock D, Simons RK, et al. Dalteparin versus enoxaparin for venous thromboembolism prophylaxis in acute spinal cord injury and major orthopedic trauma patients: 'DETECT' trial. J Trauma. 2007; 62(5):1075-81.
5. Michalis LK, Katsouras CS, Papamichael N, Adamides K, Naka KK, Goudevenos J, et al. Enoxaparin versus tinzaparin in non-ST-segment elevation acute coronary syndromes: the EVET trial. Am Heart J. 2003; 146(2):304-10.
6. Chiou-Tan FY, Garza H, Chan KT, Parsons KC, Donovan WH, Robertson CS, et al. Comparison of dalteparin and enoxaparin for deep venous thrombosis prophylaxis in patients with spinal cord injury. Am J Phys Med Rehabil. 2003; 82(9):678-85.
7. Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO, et al. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e691S-736S.
8. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med. 1993;153(13):1557-62.
9. Schulman S, Kearon C, Haemostasis IST. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005;3(4):692-4.
10. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959;22(4):719-48.
11. Roach RE, Lijfering WM, Rosendaal FR, Cannegieter SC, le Cessie S. Sex difference in risk of second but not of first venous thrombosis: paradox explained. Circulation. 2014;129(1):51-6.
12. van Dongen CJ, van den Belt AG, Prins MH, Lensing AW. Fixed dose subcutaneous low molecular weight heparins versus adjusted dose unfractionated heparin for venous thromboembolism. Cochrane Database Syst Rev. 2004:CD001100.
13. van Dongen CJ, MacGillavry MR, Prins MH. Once versus twice daily LMWH for the initial treatment of venous thromboembolism. Cochrane Database Syst Rev. 2005:CD003074.
14. Trujillo-Santos J, Bergmann JF, Bortoluzzi C, Lopez-Reyes R, Giorgi-Pierfranceschi M, Lopez-Saez JB,et al. Once versus twice daily enoxaparin for the initial treatment of acute venous thromboembolism. J Thromb Haemost 2017;15(3):429–38.
15. Charbonnier BA, Fiessinger JN, Banga JD, Wenzel E, d'Azemar P, Sagnard L. Comparison of a once daily with a twice daily subcutaneous low molecular weight heparin regimen in the treatment of deep vein thrombosis. FRAXODI group. Thromb Haemost. 1998;79(5):897-901.

SUPPLEMENTARY

Table 1. Bleeding events associated with LMWH treatment

	Follow-up (years)	Events	Cumulative incidence per 1000	Incidence rate per 100 person-years
All venous thrombotic events				
Nadroparin o.d.	151	11	2.07 (1.11-3.75)	7.3 (3.8-12.7)
Nadroparin b.i.d.	59	7	3.37 (1.48-7.10)	11.9 (5.2-23.5)
Tinzaparin o.d.	82	9	2.70 (1.33-5.20)	11.0 (5.4-20.1)
Enoxaparin	6	1	3.79 (0.00-23.34)	16.7 (8.3-82.2)
Dalteparin o.d.	40	4	2.06 (0.60-5.50)	10.0 (31.8-24.1)

CHAPTER 7

Control of anticoagulation with vitamin K antagonists: overestimation of median time in therapeutic range when assessed by linear interpolation

Thrombosis and Haemostasis. 2016;116:679-86

Joseph S. Biedermann[1,2*], Antonius M.H.P. van den Besselaar[3*], Felix J.M. van der Meer[3], Henk J. Adriaansen[4], Frank W.G. Leebeek[1], Marieke J.H.A. Kruip[2,3]

* = *First two authors contributed evenly*

[1] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[2] Star-Medical Diagnostic Center, Rotterdam, the Netherlands
[3] Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, the Netherlands
[4] Department of Clinical Chemistry and Laboratory Hematology, Gelre Hospital, Apeldoorn, the Netherlands

SUMMARY

Patients receiving vitamin K antagonists are monitored by regular assessment of the International Normalized Ratio (INR). There are two popular methods for therapeutic control of anticoagulation in patient groups: 1) Time in Therapeutic Range (TTR) assessed by linear interpolation of successive INR measurements; 2) the cross-sectional proportion (CSP) of all patients' last INRs within range. The purpose of the present study is to compare the two methods using data from 53 Dutch Thrombosis Centres and to develop a semi-quantitative model for TTR based on different types of INR change. Different groups of around 400,000 patients in four consecutive years were evaluated: patients in the induction phase, short-term, long-term, low-target range, high-target range, receiving either acenocoumarol or phenprocoumon, and performing self-management. Each Centre provided TTR and CSP results for each patient group. TTR and CSP were compared using the Wilcoxon signed-rank test. Separately, we analysed the relationship between consecutive INR results regarding in or out of range and their frequency of occurrence in patients of two different cohorts. Good correlation was observed between TTR and CSP (correlation coefficient 0.694–0.950 in low-target range). In long-term acenocoumarol patients (low-target range) the median TTR was significantly higher than CSP (80.0 % and 78.7 %, respectively; $p<0.001$). In long-term phenprocoumon patients (low-target range) there was no significant difference between median TTR (83.0 %) and median CSP (82.6 %). In conclusion, the correlation between TTR assessed by linear interpolation and CSP was good. TTR assessed by linear interpolation was higher than CSP in patients on acenocoumarol.

INTRODUCTION

Effectiveness and safety of treatment with vitamin K antagonists (VKA) depends on the intensity and quality of control of anticoagulation (1,2). During VKA treatment, the prothrombin time expressed as international normalised ratio (INR) is monitored and VKA doses are adjusted if necessary in order to achieve INR values within a specified therapeutic or target range. Different methods have been used to assess the level of therapeutic control: a cross-sectional method based on the proportion of INR test results in range at one point in time (3), and a longitudinal method based on linear interpolation of successive INR measurements to obtain the proportion of person-time spent in range which is referred to as TTR (time in therapeutic range) (4–6). The cross-sectional method is useful only for assessment of average therapeutic control for a group of patients and not for an individual patient (3). The longitudinal TTR method is useful for assessment in an individual patient as well as a group of patients. Apart from the two above-mentioned methods for assessment of the quality of anticoagulation there are other methods. One of these is the percentage of INRs within the therapeutic range (PINRR). The PINRR method utilizes the number of INRs within the target range divided by the overall number of INRs during the selected time interval (7–10). The PINRR is simple to calculate but more frequent testing in unstable patients may bias overall results (7). Other methods have been described that are modifications of the original longitudinal TTR method using imputation of INR values between pairs of INR measurements (11,12).

Observational studies have reported strong associations between group means of individual patients' TTR and reduced risks of ischaemic stroke and major bleeding in patients with atrial fibrillation (12–14). For effective stroke prevention therapy in patients with atrial fibrillation it has been recommended to use either well-controlled VKA therapy with a high percentage of time in the therapeutic range (for example, at least 70%) or one of the novel direct-acting oral anticoagulant drugs (15,16).

In the Netherlands the vast majority of patients receiving VKA are monitored by 56 Thrombosis Centres, which are members of the Dutch Federation of Thrombosis Centres. These centres achieved consensus on the intensity of anticoagulation for the various indications for treatment (17,18). Low-intensity anticoagulation (target range INR 2.5–3.5; therapeutic range INR 2.0–3.5) has been used for patients with atrial fibrillation and venous thromboembolism (VTE). High-intensity anticoagulation (target range INR 3.0–4.0; therapeutic range INR 2.5–4.0) has been used for primary and secondary prevention of arterial thromboembolism, in patients with mechanical heart valve prostheses, and in patients with recurrent VTE despite VKA treatment. The target range was set higher than the internationally advised target range to prevent

inadequate anticoagulation (6). Approximately 80 % of patients are treated with low-intensity and 20 % with high-intensity anticoagulation. In the Netherlands, two different VKA drugs are used, i. e. acenocoumarol (short acting; half-life 12 hours [h]) and phenprocoumon (long acting; half-life 160h). Acenocoumarol is used for approximately 77 % of patients, and phenprocoumon for approximately 23 % of patients. The majority of the Dutch Thrombosis Centres reported therapeutic quality control data both in terms of the cross-sectional method and in terms of the longitudinal method (19). It has not been established which of the two methods gives a better overall assessment of the control achieved by a Thrombosis Centre. The longitudinal method proposed by Rosendaal et al assumes that the INR between two measurements varies linearly from the first INR to the second INR (linear interpolation) (4). It is our hypothesis that the longitudinal method using linear interpolation of successive INR measurements is based on a simplification of the true time course and gives an overestimation of the TTR as will be explained in *Methods*. The purpose of the present study is to compare and evaluate the two methods using the data published by the Federation of the Dutch Thrombosis Centres for the years 2010, 2011, 2012 and 2013. A semi-quantitative model is presented to explain the overestimation of TTR.

METHODS

Calculation of cross-sectional proportion of INRs in the therapeutic range and proportion of time in the therapeutic range

Each Thrombosis Centre reviewed INR results for all active patients and calculated the percentage of patients for whom the last INR was within the therapeutic range at two fixed dates (March 31 and October 31) each year (17,18). The mean of the two assessmentswas used for the statistical analysis. This method is referred to as the cross-section -of-the-files method because only one INR result – of each patient is used. The percentage calculated with this method is referred to as the cross-sectional proportion (CSP).

In addition, each Thrombosis Centre used a method to estimate the percentage of time that each patient spent in the therapeutic range (TTR). In the latter method, the time between two INR measurements is divided in days and small steps of 0.1 INR over the range of the time interval (4). In this approach, the INR is treated as gradually increasing or decreasing over the time interval. This method is referred to as TTR by linear interpolation (LI). TTR(LI) is expressed as the proportion of the total person-time that lies within the range. Advantages and disadvantages of the cross-section-of-the-files method and the TTR are shown in Table 1. The cross-section-of-the-files method

Table 1. Advantages and disadvantages of methods to assess therapeutic control

Method	Advantage	Disadvantage
Cross-section-of-the-files	• Simple to calculate. • Does not make assumptions about INR between actual tests.	• Only considers one point in time. • Not useful for single patient.
TTR by linear interpolation	• Takes into account estimated time in therapeutic range. • Useful for single patient. • Allows calculation of INR specific incidence rates of adverse events.	• Calculation more difficult. • Makes assumptions about INR between actual tests.

TTR, Time in Therapeutic Range. Modified from Schmitt et al. (7).

is not useful for a single patient but can be used for a group of patients, e.g. all longterm patients monitored by one Thrombosis Centre.

CSP and TTR(LI) were assessed for various groups of patients. Patients receiving vitamin K antagonists are classified as induction phase (first 6 weeks of treatment), as short-term (longer than 6 weeks up to 6 months), or as long-term (longer than 6 months). Patients' data were provided anonymously for statistical analysis. Hence the data could not be traced back to the patients' identification and informed consent was not required. Thrombosis Centres used various (commercial) computer algorithms for anticoagulant dosing (20). At least five different computer systems were used by multiple Dutch Thrombosis Centres, e. g. Portavita, TDAS, Trodis, Glims, and Tromis (19). To study the influence of the computer algorithm used we compared TTR(LI) and CSP for Portavita centres only and for the non-Portavita centres using the combined data of four years.

Statistical methods

The Wilcoxon signed-ranks test was used to compare CSP with the TTR(LI) results obtained in each year. Spearman's coefficient (rho) for bivariate correlation between CSP and TTR(LI) was calculated. In addition, the pooled results from 2010, 2011, 2012 and 2013 were analysed with the above-mentioned statistical tests. P-values of less than 0.05 were considered statistically significant. Statistical analyses were performed with SPSS 20 (IBM Corporation, Armonk, NY, USA) for Windows.

Model for INR change

Here we develop a model which may assist us to explain the difference between TTR(LI) and the true TTR. Four hypothetical examples of the time course of INR between successive measurements are shown in Figure 1. In Figure 1A, two successive measurements are both within the therapeutic range. Evidently TTR(LI) is 100 % for this case.

In reality, this patient's INR may have been out of range for some time and the true TTR is less than 100 %. In Figure 1C, two successive measurements are both out of range at the same side of the range. Evidently TTR(LI) is 0 %. In reality, this patient's INR may have been within the range for some time between measurements and the true TTR may be greater than 0 %. Figure 1B represents a situation where one measurement is within the range and the other out of range. In this case both TTR(LI) and the true TTR are between 0 and 100 %. The depicted true course in Figure 1B would give a lower TTR than TTR(LI). It should be realised that many other true courses are possible in Figure 1B but the mean true TTR of all possible courses cannot be predicted. Figure 1D represents a situation in which two successive measurements are both out of range on opposite sides of the range. The true course depicted in Figure 1D would give a lower TTR than TTR(LI). Again, there are many other possible true courses for two consecutive INR measurements on opposite sides of the range, but the mean true TTR cannot be predicted with certainty. The hypothetical examples shown in Figure 1 refer to individual patients. To assess the total effect of linear interpolation for a group of real patients, we would like to know the frequency of the different types of INR

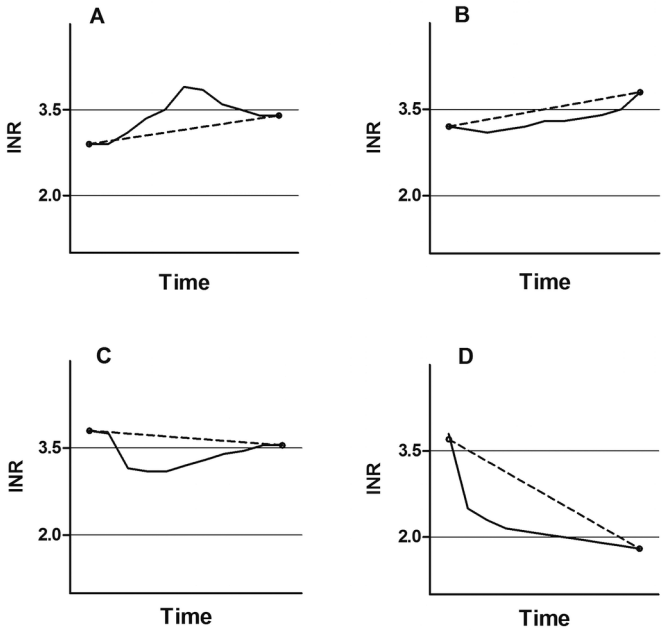


Figure 1. Schematic time-course of INR in individual patients. Four hypothetical examples are shown in panels A, B, C, and D, respectively. Horizontal lines at INR 2.0 and 3.5 represent the borders of the therapeutic range. Interrupted lines represent the linear interpolation between successive INR measurements. The drawn continuous lines represent examples of the true course of INR between successive measurements.

change. This information is not available from the annual medical reports of the Netherlands Federation of Thrombosis Services (19) and was obtained from a previous study (see below).

Assessment of frequency of INR change

Recently, a retrospective cohort study was performed using the data from the anticoagulation clinic of the Star-Medical Diagnostic Center at Rotterdam (21). We used the data from this study to assess the relative frequency of the different types of INR change in each patient. The study population consisted of two independent cohorts that were followed up for one year. One cohort was monitored using a laboratory method for venous plasma (STA Hepato Quick) and the other cohort was monitored using a point-of-care method for capillary blood (CoaguChek XS Pro). 1555 Patients in the first cohort and 1589 patients in the second cohort were treated with acenocoumarol (therapeutic range: INR 2.0–3.5). The number of INR measurements in the aforementioned patients was 30,003 and 33,060, respectively. We assessed the relationships between consecutive INR results with respect to in or out of range in each patient and calculated their frequency of occurrence. Finally, we added the numbers of the different categories of INR change for all patients in each cohort to calculate the frequency of each category of change.

RESULTS

The total number of patients monitored by the members of the Dutch Federation of Thrombosis Centres was 398,312 in 2010, 408,869 in 2011, 417,594 in 2012 and 438,411 in 2013 (19). The number of treated patients per year by each Thrombosis Centre varied from approximately 600 to approximately 27,000 (19). Table 2 shows some characteristics of the patients monitored by the members of the Dutch Federation of Thrombosis Centres. The majority of patients were treated for arterial indications (e.g. atrial fibrillation). In the period 2010–2013 there was a steady increase not only of the absolute number of patients treated for atrial fibrillation, but also as a percentage of all arterial indications.

Comparison of CSP and TTR(LI)

The majority of Thrombosis Centres provided results for CSP and TTR(LI) calculated with their own computer algorithms. The median CSP and TTR(LI) results stratified by treatment duration are shown in Table 3. In almost all cases, the median TTR(LI) was higher than the median CSP. The results shown in Table 3 were obtained irrespective

Table 2. Indications for Vitamin K antagonist treatment

Year	N	All venous indications	All arterial indications	Various arterial indications		
				Atrial fibrillation	Mechanical heart valve protheses	Other arterial indications
2010	58	15.9 ± 3.5	84.1 ± 3.5	69.3 ± 4.6	7.7 ± 1.7	23.0
2011	58	16.3 ± 3.9	83.7 ± 3.9	72.3 ± 4.6	7.4 ± 1.7	20.3
2012	55	15.2 ± 3.1	84.8 ± 3.1	74.0 ± 4.6	7.2 ± 1.8	18.8
2013	54	15.8 ± 3.2	84.2 ± 3.2	76.6 ± 3.9	6.7 ± 1.6	16.7

Mean percentages of patients with venous indications, arterial indications, atrial fibrillation (in % of arterial indications), mechanical heart valve prostheses (in % of arterial indications), and other arterial indications (e. g. coronary disease and surgery, cardiomyopathy, cerebral embolism). N is the number of Thrombosis Centres. SD is the between-centre standard deviation.

Table 3. Therapeutic control in patients by duration of treatment

Treatment duration	Year	*N*	Therapeutic Range: INR 2.0 – 3.5				Therapeutic Range: INR 2.5 – 4.0			
			CSP (%)	TTR_{LI} (%)	Wilcoxon	Correlation coefficient	CSP (%)	TTR_{LI} (%)	Wilcoxon	Correlation coefficient
Induction	2010	48	62.5	67.5	*P*= 0.000	0.766	55.6	58.8	*P*= 0.001	0.552
	2011	52	65.4	68.5	*P*= 0.000	0.769	57.8	58.1	*P*= 0.109	0.350
	2012	53	65.0	70.0	*P*= 0.000	0.778	57.1	59.3	*P*= 0.102	0.282
	2013	52	65.0	68.3	*P*= 0.000	0.694	55.8	58.0	*P*= 0.201	0.362
	All	205	64.7	68.3	*P*= 0.000	0.765	56.7	58.9	*P*= 0.000	0.380
Short-term	2010	48	74.4	77.0	*P*= 0.001	0.793	65.4	69.2	*P*= 0.001	0.660
	2011	52	76.4	78.6	*P*= 0.001	0.826	66.3	69.8	*P*= 0.001	0.605
	2012	53	77.8	78.9	*P*= 0.002	0.851	71.0	70.9	*P*= 0.405	0.536
	2013	52	77.2	78.1	*P*= 0.008	0.847	65.0	68.5	*P*= 0.013	0.555
	All	205	76.2	78.0	*P*= 0.000	0.833	66.6	69.8	*P*= 0.000	0.597
Long-term	2010	48	77.9	78.9	*P*= 0.000	0.908	72.7	74.0	*P*= 0.000	0.771
	2011	52	79.8	80.8	*P*= 0.000	0.856	73.2	75.3	*P*= 0.000	0.894
	2012	53	80.3	81.5	*P*= 0.000	0.900	74.2	75.0	*P*= 0.000	0.890
	2013	52	80.4	81.0	*P*= 0.019	0.950	73.3	75.0	*P*= 0.000	0.849
	All	205	79.9	80.9	*P*= 0.000	0.910	73.4	75.0	*P*= 0.000	0.862

The median values of CSP and TTR(LI) are given. N is the number of Thrombosis Centres in each year for each therapeutic range or the total number of comparisons between CSP and TTR over all the years.

of the type of VKA used. The median CSP and TTR(LI) for long-term patients on either acenocoumarol or phenprocoumon are given in Table 4. For acenocoumarol, the median TTR(LI) was always significantly higher than the median CSP. As an example of the correlation between TTR(LI) and CSP, a scatterplot for long-term patients receiving acenocoumarol is shown in Figure 2. Practically all Thrombosis Centres had TTR(LI) and CSP values higher than 70%. For phenprocoumon, in seven out of eight comparisons during the years 2010-2013, differences between TTR(LI) and CSP were not significant (Table 4). Several Thrombosis Centres had only few long-term patients on phenprocoumon. To avoid conclusions based on inclusion of centres with few patients, a separate analysis was performed of centres with more than 1000 long-term patients per year in the 2.0 -3.5 INR therapeutic range. This analysis showed that TTR(LI) was significantly greater than CSP for the acenocoumarol patients (Table 4). Separately, we selected 9-12 centres with more than 1000 long-term phenprocoumon patients. TTR(LI) and CSP were not significantly different for the selected centres (Table 4).

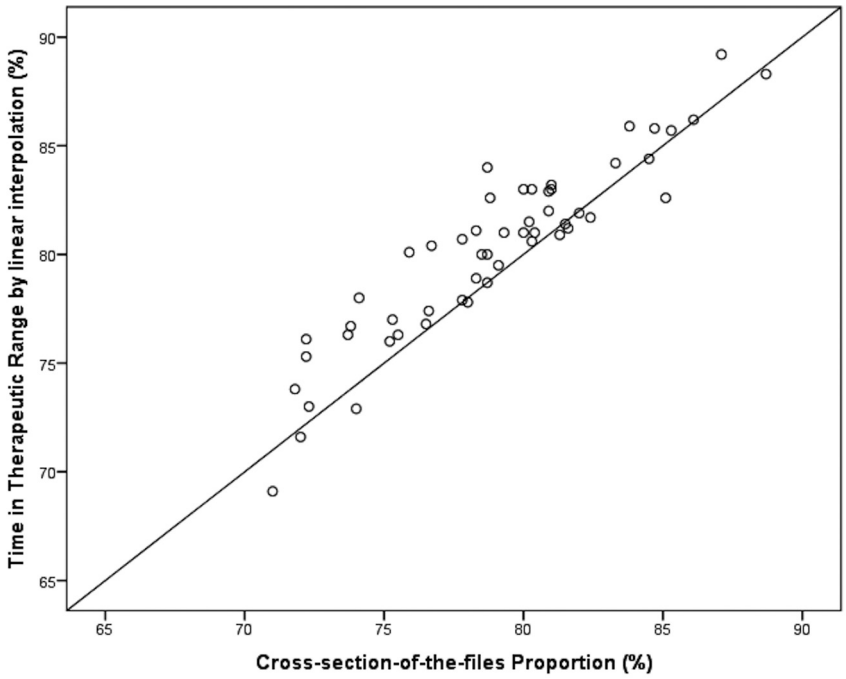


Figure 2. Scatterplot of TTR(LI) (%) versus CSP (%) . Data of long-term patients receiving acenocoumarol are shown. Therapeutic range: INR 2.0 – 3.5. Year: 2012. Each symbol represents the assessments of one Thrombosis Centre. The number of Thrombosis Centres is 53. Spearman's coefficient of correlation is 0.915. The line of identity (Y = X) is shown for comparison.

Table 4. Therapeutic control in long-term patients by type of vitamin K antagonist

Vitamin K antagonist	Year	Therapeutic Range: INR 2.0 – 3.5					Therapeutic Range: INR 2.5 – 4.0				
		N	CSP (%)	TTR_{LI} (%)	Wilcoxon	Corr. Coeff.	*N*	CSP (%)	TTR_{LI} (%)	Wilcoxon	Corr. Coeff.
Acenocoumarol	2010	48	76.6	78.0	*P*= 0.000	0.795	48	71.2	73.7	*P*= 0.000	0.626
	2011	52	79.1	80.2	*P*= 0.000	0.807	52	71.7	74.4	*P*= 0.000	0.667
	2012	53	78.8	80.9	*P*= 0.000	0.915	53	72.7	74.4	*P*= 0.000	0.814
	2013	52	79.9	80.2	*P*= 0.008	0.948	52	72.0	74.0	*P*= 0.001	0.870
	All	205	78.7	80.0	*P*= 0.000	0.876	205	71.8	74.3	*P*= 0.000	0.741
	All*	177	78.3	79.5	*P*= 0.000	0.893	39	73.0	75.0	*P*= 0.000	0.913
Phenprocoumon	2010	48	81.6	82.0	*P*= 0.948	0.695	48	77.6	78.0	*P*= 0.383	0.598
	2011	52	82.3	82.5	*P*= 0.425	0.758	52	78.9	79.0	*P*= 0.750	0.550
	2012	53	83.9	83.7	*P*= 0.296	0.792	53	79.1	80.2	*P*= 0.035	0.653
	2013	52	83.2	83.1	*P*= 0.545	0.860	52	77.8	78.2	*P*= 0.332	0.714
	All	205	82.6	83.0	*P*= 0.757	0.817	205	78.4	79.0	*P*= 0.249	0.626
	All*	42	83.8	83.8	*P*= 0.756	0.924	-	-	-	-	-

The median values of CSP and TTR(LI) are given. N is the number of Thrombosis Centres in each year or the total number of comparisons between CSP and TTR over all the years. A separate analysis was performed for Thrombosis Centres with more than 1000 patients (All*). *Only Thrombosis Centres with more than 1000 patients.

In the vast majority of the Thrombosis Centres, a proportion of the patients performed self-management using a point-of-care whole blood monitor for INR determination. This proportion varied from 2% to 15% of the patients. Patient self-management means that the patients are enabled to measure their own INR, to interpret the result, and to make adjustments to their VKA dosage by themselves (22). Patients who measure their own INR but do not make dosage adjustments are considered as regular patients and their results are included in Tables 3 and 4. CSP and TTR(LI) for self-management patients are given in Table 5. TTR(LI) was significantly higher than CSP. The results shown in Table 5 were obtained irrespective of the type of VKA used.

In most cases, the correlation coefficients between TTR(LI) and CSP were higher for the low target patients (Therapeutic range: INR 2.0 – 3.5) than for the high target (Therapeutic range: INR 2.5 – 4.0). There was a trend of increasing correlation coefficients between TTR(LI) and CSP with increasing treatment duration (Table 3).

Different dosing algorithms were used by the Thrombosis Centres. Of all computer algorithms, the "Portavita" system was used by approximately 26% (in 2010) to 44% (in 2013) of centres. We compared TTR(LI) and CSP for Portavita centres only and for the

non-Portavita centres and observed similar differences (not shown), suggesting that findings were independent of the computer dosing algorithm used.

Assessment of frequency of INR change

Table 6 shows the nine different categories of INR change between two consecutive measurements in patients on acenocoumarol (therapeutic range: INR 2.0 – 3.5) in a cohort study (21). For each patient and for each pair of consecutive INR measurements, the category of change was determined. Then the total number and proportion of cases in each category was determined. The proportion of paired measurements within the therapeutic range was 52.4% in the first cohort and 47.6% in the second. The proportion of paired measurements for which both INRs were out of the therapeutic range on the same side of the range was small (7.7% and 8.5%, respectively). As expected, the number of cases in which INRs crossed a border of the therapeutic range in one direction was similar to the number of cases in which INRs crossed the same border in the opposite direction.

Table 5. Therapeutic control in long-term patients performing self-management

Year	*N*	Therapeutic Range: INR 2.0 – 3.5				Therapeutic Range: INR 2.5 – 4.0			
		CSP (%)	TT_{LI} (%)	Wilcoxon	Correlation coefficient	CSP (%)	TT_{LI} (%)	Wilcoxon	Correlation coefficient
2010	32	80.3	82.8	*P*= 0.000	0.855	77.2	79.4	*P*= 0.002	0.519
2011	40	81.6	83.3	*P*= 0.000	0.566	76.9	79.6	*P*= 0.000	0.531
2012	45	82.7	84.7	*P*= 0.000	0.819	77.9	80.3	*P*= 0.000	0.702
2013	46	82.2	84.1	*P*= 0.000	0.837	77.6	79.4	*P*= 0.002	0.669
All	163	81.9	83.8	*P*= 0.000	0.757	77.6	79.9	*P*= 0.000	0.617

The median values of CSP and TTR(LI) are given. *N* is the number of Thrombosis Centres in each year for each therapeutic range or the total number of comparisons between CSP and TTR over all the years.

Table 6. Number of INR changes between two consecutive measurements in acenocoumarol patients with therapeutic range INR 2.0 – 3.5

Type of INR change between two consecutive measurements		**STA Hepato Quick (Cohort #1)**	**CoaguChek XS Pro (Cohort #2)**
First measurement	Second measurement	Number (% of total)	Number (% of total)
2.0 < INR < 3.5	2.0 < INR < 3.5	14,916 (52.4 %)	14,990 (47.6 %)
2.0 < INR < 3.5	INR > 3.5	3,500 (12.3 %)	4,049 (12.9 %)
2.0 < INR < 3.5	INR < 2.0	1,713 (6.0 %)	2,121 (6.7 %)
INR < 2.0	2.0 < INR < 3.5	1,939 (6.8 %)	2,366 (7.5 %)
INR < 2.0	INR < 2.0	754 (2.7 %)	868 (2.8 %)
INR < 2.0	INR > 3.5	370 (1.3 %)	593 (1.9 %)
INR > 3.5	2.0 < INR < 3.5	3,327 (11.7 %)	3,904 (12.4 %)
INR > 3.5	INR < 2.0	509 (1.8 %)	791 (2.5 %)
INR > 3.5	INR > 3.5	1,420 (5.0 %)	1,789 (5.7 %)

The frequency of INR changes is given in percent. Two independent cohorts were analysed, each cohort being monitored with a different prothrombin time reagent (STA Hepato Quick and CoaguChek XS Pro, respectively).

DISCUSSION

The present study was an analysis of data published by the Dutch Federation of Thrombosis Centres. The purpose of our study was to compare TTR(LI) with CSP using data from the majority of VKA users in the Netherlands. In general, there was good correlation between the two methods to evaluate control of anticoagulation in groups of patients, which is in agreement with a previous single-centre study (6). TTR(LI) was slightly higher than CSP in most comparisons. The greatest difference between the two methods was observed for patients in the induction phase of treatment (Table 3) and the smallest difference for long-term patients receiving phenprocoumon (Table 4).
Our working hypothesis is that the probability to find an individual patient's INR at any time in the therapeutic range is equal to the true proportion of time spent in the therapeutic range. Therefore, CSP is expected to be equal to TTR. Why then is TTR(LI) slightly greater than CSP? It should be realized that the true TTR cannot be determined and that TTR(LI) is an approximation of the true TTR, because the assumed linear INR change is an approximation of the true time-course. In contrast, CSP is calculated independently from any model of the INR time-course in individual patients. CSP is always calculated for one point in time. TTR(LI) is calculated for a certain time interval, e.g. one year. The patients used for calculation of CSP were not completely the same as the patients used for calculation of TTR(LI) because some patients included in TTR(LI) may have stopped anticoagulation treatment before CSP was calculated. It is unlikely

that the difference in individual patients could completely explain the consistently observed pattern of differences between CSP and TTR(LI).

In this paper we present a model for the change of INR with regard to the calculation of TTR(LI). The relative frequency of the different types of INR change shown in Figure 1 may help us to explain the difference between TTR(LI) and CSP. Since the majority of INR measurements is within the therapeutic range, situation 1A occurs more often than situation 1C, which is confirmed by observed frequencies in the cohort study (Table 6). INR changes within the therapeutic range (situation 1A) that may overestimate TTR(LI) occur approximately 5-7 times more frequently than INR changes that may underestimate TTR(LI) (Table 6). As a result, the true TTR summed over all patients will be less than the TTR(LI) and therefore calculation of TTR(LI) will result in an overestimation of the true TTR. A limitation of our semi-quantitative model is that we cannot predict the exact magnitude of the difference between TTR(LI) and the true TTR. The difference between TTR(LI) and the true TTR will depend on the magnitude of INR variation over time. If the within-subject INR variation is increased, there will be an increased probability that the INR deviates from a straight-line path between successive measurements. INR variation will be greater in the induction phase of treatment than in patients who are treated for more than 6 months (i.e. long-term treatment). Therefore, the difference between TTR(LI) and the true TTR will be greater in the induction phase than in the long-term steady state of treatment. Other investigators concluded that the validity of the linear interpolation method could be improved by using a hybrid method that takes into account potential effects of dosage modifications when INRs are far out of the target range (11).

INR variation is also greater in patients receiving the short-acting acenocoumarol than in patients receiving the long-acting phenprocoumon (23). This can explain why the difference between TTR(LI) and CSP is smaller for phenprocoumon than for acenocoumarol. When there is more variation of INR, there is greater probability that the INR is out of therapeutic range between two successive measurements which are both within the range. In long-term patients receiving phenprocoumon, the variation of INR is smaller compared to acenocoumarol and the linear interpolation may be a good approximation of the true time course of INR.

Our study is limited to patients treated with acenocoumarol and phenprocoumon. Warfarin (half-life 40 h) is not used in the Netherlands. Other investigators compared therapeutic control in warfarin treated patients to that in acenocoumarol treated patients using the cross-sectional method (24). They reported that the cross-sectional method did not show any difference between the two drugs (24). Furthermore, daily fluctuations of factor VII levels were similar with both drugs (24). It seems that warfarin is more similar to acenocoumarol than to phenprocoumon with regard to the pharmacodynamics and

INR variability. Further studies should be performed to investigate whether the difference between CSP and TTR(LI) is also observed in warfarin patients.

The strength of our study is the large number of Thrombosis Centres, patients and INR measurements used for the analysis. Because of the large numbers, the pattern of differences between TTR and CSP could consistently be evaluated with adequate power thereby reducing the risk of observations by chance. A limitation of our study is that the number of patients varied between the individual Thrombosis Centres but nevertheless the data from each centre were treated with equal weight. It has been stated that CSP determined for few patients may be unrealistic (5). However, when our analysis was limited to centres with a large number of patients, i.e. those with at least 1000 long-term patients per year, similar differences between CSP and TTR(LI) were obtained and the statistical significance of the differences did not change. A second limitation of our study is that we cannot perform an analysis of the same data with other methods such as the PINRR (8-10) or a hybrid method that takes into account potential effects of dosage modifications when INRs are far out of the target range (11,12). We would like to emphasise that our data apply to the evaluation of the quality of anticoagulation of a Thrombosis Centre and cannot be applied to the single patient. Despite these limitations it is reassuring that in nearly all Dutch Thrombosis Centres the median of percentage long-term patients within the low-intensity therapeutic range was at least 70%. The high overall median TTR(LI) for long-term patients (Table 3) with respect to other reports (12, 25-27) may be explained in part by the use of long-acting phenprocoumon, and in part by the slightly wider therapeutic range (e.g. INR 2.0-3.5, rather than 2.0-3.0). By comparing Tables 3 and 5, it can be concluded that therapeutic quality of long-term patients performing self-management is at least as good or even better than the quality of long-term patients managed by the regular system, in agreement with a previous study (28).

In conclusion, we have shown that there is good correlation between TTR assessed by linear interpolation and CSP obtained by Dutch Thrombosis Centres. Our model, which is based on observed frequencies of the relationship between consecutive INR measurements, predicts that the linear interpolation method overestimates the true TTR. The difference between TTR and CSP is greater in the induction phase than in the long-term steady state phase of anticoagulation and is greater in acenocoumarol patients than in phenprocoumon patients.

REFERENCES

1. Connolly SJ, Pogue J, Eikelboom J, Flaker G, Commerford P, Franzosi MG, et al. Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. Circulation 2008;118(20):2029-37.
2. Poli D, Antonucci E, Testa S, Tosetto A, Agena W, Palareti G, et al. Bleeding risk in very old patients on vitamin K antagonist treatment. Results of a prospective collaborative study on elderly patients followed by Italian centres for anticoagulation. Circulation 2011;124(7):824-9.
3. Van den Besselaar AM. Recommended method for reporting therapeutic control of oral anticoagulant therapy. Control of Anticoagulation Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost 1990;63(2):316-7.
4. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E. A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost 1993;69(3):236-9.
5. Azar AJ, Deckers JW, Rosendaal FR, van Bergen PF, van der Meer FJ, Jonker JJ, et al. Assessment of therapeutic quality control in a long-term anticoagulant trial in post-myocardial infarction patients. Thromb Haemost 1994;72(3):347-51.
6. Bezemer ID, Roemer WH, Penning-van Beest FJ, van Eekelen E, Kramer MH. INR control calculation: comparison of Dutch and international methods. Neth J Med 2013;71(4):194-8.
7. Schmitt L, Speckman J, Ansell J. Quality assessment of anticoagulation dose management: comparative evaluation of measures of time-in-therapeutic range. J Thromb Thrombolysis 2003;15(3):213-6.
8. Chan PH, Li WH, Hai JJ, Chan EW, Wong IC, Tse HF, et al. Time in therapeutic range and percentage of international normalized ratio in the therapeutic range as a measure of quality of anticoagulation control in patients with atrial fibrillation. Can J Cardiol 2016;32(10): 1247.e23–1247.e28.
9. Caldeira D, Cruz I, Morgado G, Stuart B, Gomes AC, Martins C, et al. Is the time in therapeutic range using the ratio of tests equivalent to the Rosendaal method? Blood Coagul Fibrinolysis 2015;26(8):972-6.
10. Abumuaileq RR, Abu-Assi E, Raposeiras-Roubin S, Lopez-Lopez A, Redondo-Dieguez A, Rodriguez-Manero M, et al. Evaluation of SAMe-TT2R2 risk score for predicting the quality of anticoagulation control in a real-world cohort of patients with non-valvular atrial fibrillation on vitamin-K antagonists. Europace 2015;17(5):711-7.
11. Hutten BA, Prins MH, Redekop WK, Tijssen JG, Heisterkamp SH, Buller HR. Comparison of three methods to assess therapeutic quality control of treatment with vitamin K antagonists. Thromb Haemost 1999;82(4):1260-3.
12. Singer DE, Hellkamp AS, Yuan Z, Lokhnygina Y, Patel MR, Piccini JP, et al. Alternative calculations of individual patient time in therapeutic range while taking warfarin: results from the ROCKET AF trial. J Am Heart Assoc 2015;4:e001349.
13. White HD, Gruber M, Feyzi J, Kaatz S, Tse HF, Husted S, et al. Comparison of outcomes among patients randomized to warfarin therapy according to anticoagulant control: results from SPORTIF III and V. Arch Intern Med 2007;167(3):239-45.
14. Wallentin L, Yusuf S, Ezekowitz MD, Alings M, Flather M, Franzosi MG, et al. Efficacy and safety of dabigatran compared with warfarin at different levels of international normalised ratio control for stroke prevention in atrial fibrillation: an analysis of the RE-LY trial. Lancet 2010;376(9745):975-83.
15. De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, et al. New oral anticoagulants in atrial fibrillation and acute coronary syndromes. ESC Working Group on Thrombosis – Task Force on Anticoagulants in Heart Disease Position Paper. J Am Coll Cardiol 2012;59(16):1413-25.

16. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, et al. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation – Developed with the special contribution of the European Heart Rhythm Association. Eur Heart J 2012;33(21):2719-47.
17. Breukink-Engbers WG. Monitoring therapy with anticoagulants in The Netherlands. Sem Thromb Hemost 1999;25(1):37-42.
18. Van Geest-Daalderop JH, Sturk A, Levi M, Adriaansen HJ. Extent and quality of anti-coagulation treatment with coumarin derivatives by the Dutch Thrombosis Services. Ned Tijdschr Geneeskd 2004;148(15):730-5.
19. Netherlands Federation of Thrombosis Services. Summary medical annual reports. Available at http://www.fnt.nl/.
20. Van Leeuwen Y, Rombouts EK, Kruithof CJ, van der Meer FJ, Rosendaal FR. Improved control of oral anticoagulant dosing: a randomized controlled trial comparing two computer algorithms. J Thromb Haemost 2007;5(8):1644-9.
21. Biedermann JS, van Rein N, van den Besselaar AM, Buhre PN, de Maat MP, van der Meer FJ, et al. Impact of point-of-care INR monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: a cohort study. J Thromb Haemost 2016;14(4):695-703.
22. Braun S, Spannagl M, Völler H. Patient self-testing and self-management of oral anticoagulation. Anal Bioanal Chem 2009;393(5):1463-71.
23. Fihn SD, Gadisseur AA, Pasterkamp E, van der Meer FJ, Breukink-Engbers WG, Geven-Boere LM, et al. Comparison of control and stability of oral anticoagulant therapy using acenocoumarol versus phenprocoumon. Thromb Haemost 2003;90(2):260-6.
24. Barcellona D, Vannini ML, Fenu L, Balestrieri C, Marongiu F. Warfarin or acenocoumarol: which is better in the management of oral anticoagulants? Thromb Haemost 1998;80(6):899-902.
25. Ansell J, Hollowell J, Pengo V, Martinez-Brotons F, Caro J, Drouet L. Descriptive analysis of the process and quality of oral anticoagulation management in real-life practice in patients with chronic non-valvular atrial fibrillation: the international study of anticoagulation management (ISAM). J Thromb Thrombolysis 2007;23(2):83-91.
26. Wan Y, Henegan C, Perera R, Roberts N, Hollowell J, Glasziou P, et al. Anticoagulation control and prediction of adverse events in patients with atrial fibrillation. A systematic review. Circ Cardiovasc Qual Outcomes 2008;1(2):84-91.
27. Fitzmaurice DA, Accetta G, Haas S, Kayani G, Lucas Luciardi H, Misselwitz F, et al. Comparison of international normalized ratio audit parameters in patients enrolled in GARFIELD-AF and treated with vitamin K antagonists. Br J Haematol 2016;174(4):610-23.
28. Gadisseur AP, Breukink-Engbers WG, Van der Meer FJ, van den Besselaar AM, Sturk A, Rosendaal FR. Comparison of the quality of oral anticoagulant therapy through patient self-management and management by specialized anticoagulation clinics in the Netherlands: a randomized clinical trial. Arch Intern Med 2003;163(21):2639-46.

CHAPTER 8

Predictors of oral cavity bleeding and clinical outcome after dental procedures in patients on vitamin K antagonists: a cohort study

Thrombosis and Haemostasis 2017;117:1432-9

Joseph S. Biedermann[1,2*], Willem M.H. Rademacher[3*], Hendrika C.A.M. Hazendonk[1], Denise E. van Diermen[3], Frank W.G. Leebeek[1], Frederik R. Rozema[3], Marieke J.H.A. Kruip[1,2]

* = *First two authors contributed evenly*

[1] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[2] Star-Medical Diagnostic Center, Rotterdam, the Netherlands
[3] Department of Oral Medicine, Acadamic Center for Dentistry, Amsterdam, the Netherlands

SUMMARY

Patients on vitamin K antagonists (VKA) often undergo invasive dental procedures. International guidelines consider all dental procedures as low-risk procedures, while bleeding risk may differ between standard low-risk (e.g. extraction 1-3 elements) and extensive high-risk (e.g. extraction of >3 elements) procedures. Therefore current guidelines may need refinement. In this cohort study, we identified predictors of oral cavity bleeding (OCB) and evaluated clinical outcome after low-risk and high-risk dental procedures in patients on VKA. Perioperative management strategy, procedure risk, and 30-day outcomes were assessed for each procedure. We identified 1845 patients undergoing 2004 low-risk and 325 high-risk procedures between 2013 and 2015. OCB occurred after 67/2004 (3.3%) low-risk and 21/325 (6.5%) high-risk procedures (P=0.006). In low-risk procedures, VKA continuation with tranexamic acid mouthwash was associated with a lower OCB risk compared to continuation without mouthwash [OR=0.41, 95%CI 0.23-0.73] or interruption with bridging [OR=0.49, 95%CI 0.24-1.00], and a similar risk as interruption without bridging [OR=1.44, 95%CI 0.62-3.64]. In high-risk procedures, VKA continuation was associated with an increased OCB risk compared to interruption [OR=3.08, 95%CI 1.05-9.04]. Multivariate analyses revealed bridging, antiplatelet therapy, and a supratherapeutic or un-objectified INR before the procedure as strongest predictors of OCB. Non-oral cavity bleeding (NOCB) and thromboembolic event (TE) rates were 2.1% and 0.2%. Bridging therapy was associated with a two-fold increased risk of NOCB [OR=1.93, 95%CI 1.03-3.60], but not with lower TE rates. In conclusion, predictors of OCB were mostly related to perioperative management and differed between low-risk and high-risk procedures. Perioperative management should be differentiated accordingly.

INTRODUCTION

Due to the high prevalence of cardiovascular disease worldwide, millions of people currently receive oral anticoagulants such as vitamin K antagonists (VKA). Patients on VKA often require invasive dental procedures for which they require periprocedural VKA management. The bleeding risk after dental procedures in these patients is higher than in individuals without VKA therapy (1). Various studies have compared different management strategies in order to minimise the risk of oral cavity bleeding after dental procedures, without increasing the risk of thromboembolic complications (2-4). However, most of these studies included only small numbers of patients and were underpowered to detect differences in bleeding rates between different management strategies. Current guidelines and guidance documents have suggested that VKA therapy can safely be continued with co-administration of a local pro-haemostatic agent (e.g. tranexamic acid [TXA] mouthwash) during low bleeding risk dental procedures (5-8). However, these guidelines do not differentiate between low and high bleeding risk dental procedures in their recommendations on VKA-management. Differentiating into these categories may lead to clearer and safer perioperative strategies. The use of preoperative international normalised ratio (INR) values in these guidelines and standardised VKA management probably makes the bleeding risk in patients on warfarin similar to that of patients on acenocoumarol or phenprocoumon and vice versa.

The goals of the present study were to evaluate, in a real world setting, VKA management and clinical outcome after low-risk and high-risk dental procedures in patients on VKA, and to identify predictors of oral cavity bleeding for both categories.

METHODS

Study Design and Setting

We used data from the anticoagulation clinic of the Star-Medical Diagnostic Centre (Rotterdam, the Netherlands). All registered dental procedures in patients on VKA between January 1, 2013 and January 1, 2015 were retrieved from the clinic's medical database. These procedures were either reported beforehand by the patients or treating physicians or in retrospect by the patients during subsequent visits to the clinic. We collected information regarding patient and dental procedure characteristics, periprocedural VKA management. A waiver for informed consent was granted on behalf of the ethics committee of the Erasmus University Medical Centre based on the observational nature of our study.

Periprocedural VKA management

In the Netherlands, patients are treated with acenocoumarol or phenprocoumon and are monitored by anticoagulation clinics. Dental practitioners consult these clinics for advice regarding periprocedural VKA management. For standard low-risk procedures (e.g. extraction or implantation 1-3 elements), VKA management is based on the guideline from the Academic Centre for Dentistry Amsterdam (ACTA) (5). This guideline classifies dental procedures as low or high bleeding risk, and states that low-risk procedures can be safely performed under VKA continuation, provided that the INR is ≤ 3.5, the wound is sutured, and a local pro-haemostatic agent (TXA-mouthwash 5.0%, 10ml 4dd for 5 days) is prescribed (5). In order to follow this protocol, the patients must report the planned dental procedure to the anticoagulation clinic at least 24 h in advance. This guideline, however, does not provide guidance on perioperative management for high-risk procedures (e.g. extraction or implantation >3 elements and orthognatic chirurgery). For these high-risk procedures, anticoagulant therapy is usually interrupted without routine TXA prescription, and bridged with low-molecular-weight heparin (LMWH) if required, in line with international recommendations (6). Regardless of the bleeding risk of the elective dental procedure, when VKA therapy is interrupted, the INR is not routinely measured at the clinic prior to the procedure as discontinuation for several days in patients treated with the short-acting acenocoumarol is sufficient to ensure adequately low INR levels.

Candidate predictors for oral cavity bleeding

Candidate predictors for oral cavity bleeding were selected beforehand based on literature and presumed clinical relevance (1,6). The following patient characteristics were analysed: age, sex, intensity of VKA treatment, type of VKA, and quality of anticoagulation control prior to the procedure defined as percentage of time in therapeutic range (TTR in %). The TTR was calculated using the Rosendaal method for each patient from 3 months until 1 week prior to the procedure (9).

Potential predictors related to periprocedural management were: concomitant exposure to antiplatelet agents (thrombocyte aggregation inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), and selective serotonin reuptake inhibitors (SSRIs)), whether VKA treatment was interrupted, whether the last INR result at the clinic was ≤3.5, whether the procedure was reported at least 24 hours in advance, and whether a valid INR-measurement was performed at the anticoagulation clinic within 72 h before the procedure. Since patients are differently managed depending on the classification as low-risk or high-risk procedure, we identified the predictors for bleeding separately according to the ACTA classification.

Study Outcomes

Our primary outcome was clinically relevant oral cavity bleeding (OCB) within 30 days after the procedure. Bleedings were considered clinically relevant if these: 1) were spontaneously reported by the patient to the anticoagulation clinic apart from planned visits, 2) required a second intervention or alteration in medication, or 3) caused hospitalisation or death. Minor bleedings such as small haematomas reported only during routine visits were not considered clinically relevant. Since patients at our thrombosis service are instructed at each visit to proactively report serious bleeding complications between visits, we considered this definition an adequate cut-off for clinically relevant bleeding with a low chance of missing these bleedings.

Secondary outcomes were: clinically relevant non-oral cavity bleedings (NOCB) (using the previously mentioned definition for clinical relevance), objectified thromboembolic complications (transient ischaemic attack, ischaemic stroke, myocardial infarction or venous thrombosis), hospitalisation (any), and all-cause mortality within 30 days. Two different investigators (JB, WR) independently classified all procedures as low-risk or high-risk and evaluated periprocedural management for each procedure. All outcome events were independently classified by physicians of the anticoagulation clinic as part of routine care and reviewed by both investigators.

Statistical analysis

Standard descriptive statistics were performed to assess differences regarding patient characteristics, procedure characteristics, and clinical outcomes. Continuous covariates were compared between groups by Student's t-test in case of a normal distribution and by Mann Whitney-U test for non-normally distributed covariates. Proportions were compared by Chi-square test. For all clinical outcomes, 30-day event rates with 95% confidence intervals (CI) were calculated in line with recommendations for reporting procedure-related outcomes (10). Univariate logistic regression analysis and multivariate backward conditional logistic regression analysis were used to identify predictors of oral cavity bleeding after low-risk and high-risk procedures. Odds ratios (OR) with 95% CI were calculated and compared between different management strategies regarding the risk of oral cavity bleeding. We performed a sensitivity analysis including only the first procedure of each patient during the study period. If the second intervention was a re-intervention, it is conceivable that this could also affect the risk of bleeding and therefore influence the results. P-value for model inclusion in the backward logistic regression models was set at p=0.10. Statistics were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Study population

In total, 2666 dental procedures were identified, performed in 2181 patients between January 2013 and January 2015. Of these, 337 (14.1%) procedures performed in 336 patients were excluded for analysis for various reasons (Figure 1). After exclusion, 2329 procedures performed in 1845 patients were included for final analysis. Of these, 2004 (86.0%) were low-risk procedures and 325 (14.0%) high-risk procedures. Most patients (n=1457, 79.0%) underwent one procedure during the study period. Procedure and patient characteristics, overall and by procedure risk, are shown in Table 1.

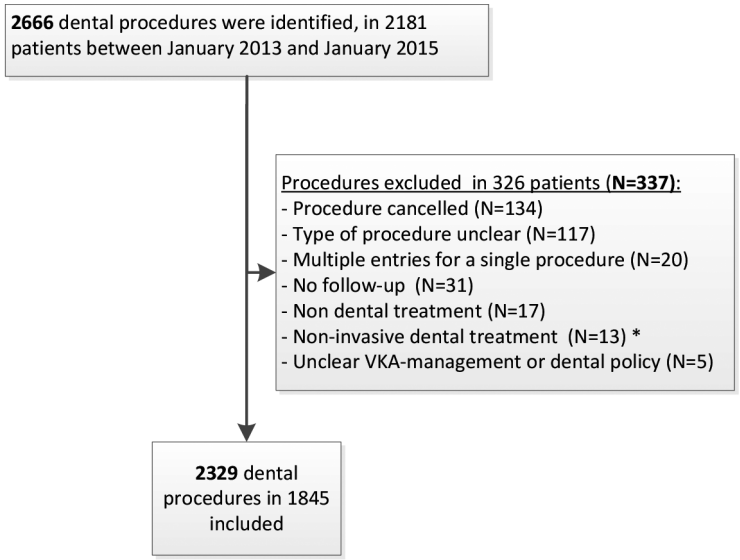


Figure 1. Study flow chart. * E.g. annual check-up, prosthesis adjustments, radiographic imaging. N= number of procedures

Low bleeding risk procedures

Of the 2004 low-risk procedures, 1540 (77.8%) were reported to the clinic at least 24 h in advance. In 1083/2004 (54.0%) procedures, a valid INR measurement was performed at the anticoagulation clinic within 72 h before the procedure. Treatment with VKA was continued in 1350/2004 (67.4%) procedures, of which 900/1350 (66.7%) with TXA mouthwash and 450/1350 (33.3%) without. Treatment with VKA was interrupted in 654/2004 procedures (32.6%), of which 246/654 (37.6%) were bridged

with LMWH. Clinically relevant oral cavity bleeding within 30 days occurred in 67/2004 low-risk procedures (3.3%, 95%CI 2.6-4.2). Oral cavity bleeding rates, ordered by procedure risk and management strategy, are shown in Table 2. Oral cavity bleeding occurred significantly more often in patients using antiplatelet therapy (16/237, 6.8%), compared to non-users (51/1767, 2.9%) [p=0.002]. Overall, the bleeding risk after continuation of VKA with TXA mouthwash was similar to VKA interruption without bridging [OR 1.44, 95%CI 0.62-3.64]. Continuation of VKA with TXA mouthwash was, however, associated with a lower bleeding risk compared to VKA continuation without TXA mouthwash [OR=0.41, 95%CI 0.23-0.73] or VKA interruption with bridging [OR=0.49, 95%CI 0.24-1.00]. When VKA therapy was interrupted (n=654), bridging was associated with an increased bleeding risk compared to forgoing bridging [OR=2.94, 95%CI 1.14-7.57] (Table 2). Sensitivity analysis revealed similar results (Table 2).

Table 1. Patient and procedure characteristics by procedure risk

	No. (%)			
	Overall (n=2329)	**Low-risk (n=2004)**	**High-risk (n=325)**	**P Value**
Characteristic				
Patient				
Age, median [IQR], y	73.0 [64.0-81.0]	73.0 [64.0-81.0]	73.0 [65.0-81.0]	0.254
Male sex	1297 (55.7)	1092 (54.5)	205 (63.1)	0.004
VKA treatment duration, median [IQR], y	4.4 [1.3-10.2]	4.4 [1.3-10.2]	4.5 [1.3-10.6]	0.537
VKA type				
Acenocoumarol	2156 (93.1)	1853 (92.5)	303 (93.2)	0.829
Phenprocoumon	173 (6.9)	151 (7.5)	22 (6.8)	
Treatment indication				
Atrial fibrillation	1442 (61.9)	1235 (61.6)	207 (63.7)	<0.001
Venous thrombosis	301 (12.9)	270 (13.5)	31 (9.5)	
Heart valve replacement	154 (6.6)	146 (7.3)	8 (2.5)	
Arterial thrombosis	418 (17.9)	341 (17.0)	77 (23.7)	
Prophylaxis	14 (0.6)	12 (0.6)	2 (0.6)	
Therapeutic INR range				
2.0-3.5	1855 (79.6)	1598 (79.7)	257 (79.1)	0.963
2.5-4.0	404 (17.3)	346 (17.3)	58 (17.8)	
Other	70 (3.0)	60 (3.0)	10 (3.1)	
TTR	82.0 [60.6-100.0]	82.9 [61.5-100.0]	77.4 [53.8-95.7]	0.004

Table 1. *Continued*

	No. (%)			
	Overall (n=2329)	**Low-risk (n=2004)**	**High-risk (n=325)**	**P Value**
Procedure				
Type				
Tooth extraction of 1-3 elements	1403 (60.2)	1403 (70.0)	-	-
Endodontic therapy	68 (2.9)	68 (3.4)	-	
Abscess incision	16 (0.7)	16 (0.8)	-	
Tooth implantation of 1-3 elements	118 (5.1)	118 (5.9)	-	
Scaling or root planning	259 (11.1)	259 (12.9)	-	
Tooth restauration	42 (1.8)	42 (2.1)	-	
Apex resection	16 (0.7)	16 (0.8)	-	
Wisdom tooth extraction	28 (1.2)	28 (1.4)	-	
Periodontal flap surgery	17 (0.7)	17 (0.8)	-	
Dental crown or tooth bridge	37 (1.6)	37 (1.8)	-	
Tooth extraction of >3 elements	296 (12.8)	-	296 (91.1)	-
Tooth implantation of >3 elements	13 (0.6)	-	13 (4.0)	
Orthognathic surgery	16 (0.7)	-	16 (4.9)	
Reported at least 24h in advance	1834 (78.7)	1540 (76.8)	294 (90.5)	<0.001
VKA interrupted for procedure	946 (40.6)	654 (32.6)	292 (89.8)	<0.001
Bridging with LMWH	397 (17.0)	246 (12.3)	151 (46.5)	<0.001
Tranexamic acid mouthwash prescribed	967 (41.5)	947 (47.3)	20 (6.2)	<0.001
Valid INR at clinic within 72h	1144 (49.1)	1083 (54.0)	61 (18.8)	<0.001
Last INR at clinic ≤ 3.5	1942 (83.4)	1658 (82.7)	284 (87.4)	0.037
Periprocedural exposure to antiplatelet drugs				
Thrombocyte aggregation inhibitors (any)	282 (12.1)	237 (11.8)	45 (13.8)	0.300
Acetylsalicylic acid	170 (7.3)	141 (7.0)	29 (8.9)	0.546
Clopidogrel	80 (3.4)	69 (3.4)	11 (3.4)	
Dipyridamol	26 (1.1)	21 (1.0)	5 (1.5)	
Prasugrel	6 (0.3)	6 (0.3)	-	
NSAID	268 (11.5)	232 (11.6)	36 (11.1)	0.793
SSRI	141 (6.1)	122 (6.1)	19 (5.8)	0.865

IQR=Interquartile range, VKA=Vitamin K Antagonist, INR=International Normalized Ratio, TTR=Time in Therapeutic Range, LMWH=Low-Molecular-Weight Heparin, NSAID=Non-steroid anti-inflammatory drug, SSRI=Selective Serotonin Reuptake Inhibitor.

Table 2. Oral cavity bleeding within 30 days by procedure risk and management strategy

	No. of bleeding / Total No. (%)					
	VKA continuation			VKA interruption		
	Without TXA	With TXA	OR (95% CI)	Without Bridging	With Bridging	OR (95% CI)
All procedures						
Low-Risk (N=2004)	26/450 (5.8%)	22/900 (2.4%)	0.41 (0.23-0.73)	7/408 (1.7%)	12/246 (4.9%)	2.94 (1.14-7.57)
High-Risk (N=325)	4/23 (17.4%)	1/10 (10.0%)	0.53 (0.05-5.43)	6/141 (4.3%)	10/151 (6.6%)	1.60 (0.56-4.51)
Overall (N=2329)	30/473 (6.3%)	23/910 (2.5%)	0.38 (0.22-0.67)	13/549 (2.4%)	22/397 (5.5%)	2.42 (1.20-4.86)
Sensitivity analysis *						
Low-Risk (N=1597)	21/365 (5.8%)	15/729 (2.1%)	0.34 (0.18-0.68)	5/315 (1.6%)	9/188 (4.8%)	3.12 (1.03-9.45)
High-Risk (N=248)	4/20 (20.0%)	0/7 (0.0%)	-	4/106 (3.8%)	9/115 (7.8%)	2.17 (0.65-7.25)
Overall (N=1845)	25/385 (6.3%)	15/736 (2.5%)	0.30 (0.16-0.58)	9/421 (2.1%)	18/303 (5.9%)	2.89 (1.28-6.53)

* First procedure from each patient. VKA=Vitamin K Antagonist, TXA=Tranexamic acid mouthwash, LMWH=Low-Molecular-Weight Heparin, OR=Odds Ratio, CI=Confidence Interval

Backward conditional modelling revealed that bridging therapy [OR=3.19, 95%CI 1.22-8.35], a missing [OR=1.90, 95%CI 1.10–3.28] or supratherapeutic INR [OR=1.75, 95%CI 0.98–3.12] before the procedure, procedures that were not reported to the clinic in advance [OR=2.60, 95%CI 1.52–4.46] and concomitant exposure to thrombocyte aggregation inhibitors [OR=2.40, 95%CI 1.33–4.32] were the factors most strongly associated with an increased risk of oral cavity bleeding (Table 3).

Table 3. Predictors of oral cavity bleeding by procedure risk

	Beta	**OR (95% CI)**
Low-risk (n=2004)		
Bridging with LMWH	1.159	3.19 (1.22 - 8.35)
Exposure to platelet aggregation inhibitor	0.874	2.40 (1.33 - 4.32)
No valid INR before procedure	0.641	1.90 (1.10 - 3.28)
Last INR at clinic >3.5	0.557	1.75 (0.98 - 3.12)
Procedure not reported in advance	0.956	2.60 (1.52 - 4.46)
VKA interruption	-0.880	0.42 (0.18 - 0.96)
Time in therapeutic range (per percent increase)	0.010	1.01 (1.00 - 1.02)
High-risk (n=325)		
Exposure to NSAID	1.411	4.10 (1.38 - 12.20)
VKA interruption	-1.992	0.14 (0.03 - 0.58)
No valid INR before procedure	1.658	5.25 (0.92 - 30.11)
Age at procedure (per year increase)	0.046	1.05 (1.00 - 1.09)
Time in therapeutic range (per percent increase)	-0.018	0.98 (0.97 - 1.00)

Backward conditional logistic regression model. OR=Odds Ratio, INR=International Normalized Ratio; NSAID=Non-Steroidal Anti-Inflammatory Drug; LMWH=Low-Molecular-Weight Heparin, VKA= Vitamin K Antagonist

High bleeding risk procedures

Of the 325 high-risk procedures, 294 (90.5%) were reported to the clinic at least 24 h in advance. Most high-risk procedures (n=296, 91.1%) were extractions of more than three elements (Table 1). VKA therapy was interrupted in 292/325 (89.8%) of these procedures, of which 151/292 (51.7%) were bridged with LMWH. Clinically relevant oral cavity bleeding within 30 days occurred in 21/325 (6.5%, 95%CI 4.3-9.7) of these procedures (Table 2). Oral cavity bleeding rates were significantly higher in patients using NSAIDs (6/36, 16.7%) compared to non-users (15/289, 5.2%) [p=0.008].

Overall, VKA continuation was associated with a significantly higher bleeding risk compared to VKA interruption [OR=3.08, 95%CI 1.05-9.04]. When VKA was interrupted, bridging with LMWH was not associated with a significantly higher bleeding risk compared to forgoing bridging [OR=1.60, 95%CI 0.56-4.51]. Sensitivity analysis revealed similar results (Table 2).

Backward conditional modelling revealed that exposure to NSAIDs [OR=4.10, 95%CI 1.38-12.20] and a missing INR before the procedure [OR=5.25, 95%CI 0.92-30.11] were associated with an increased risk of bleeding for high-risk procedures, while VKA interruption strongly lowered the risk of bleeding [OR=0.14, 95%CI 0.03-0.58] (Table 3).

Secondary clinical outcomes

Clinically relevant non-oral cavity bleeding within 30 days occurred in 50/2329 procedures (2.1%, 95%CI 1.6-2.8%). Of these bleedings, one was an intracranial bleeding (2%), four were gastrointestinal bleedings (8%), three patients reported haematuria (6%) and three bleedings were of vaginal origin (6%). All other bleedings were cutaneous bleedings (30/50, 60%) or nose bleedings (9/50, 18%).

The bleeding rates after low-risk (41/2004, 2.0%) and high-risk (9/325, 2.8%) procedures were similar [p=0.40]. Non-oral cavity bleeding occurred more often after procedures that were bridged with LMWH (14/397, 3.5%) compared to those where VKA therapy was continued or interrupted without bridging (36/1932, 1.9%) [p=0.04]. Bridging therapy was associated with an almost two-fold increased risk of non-oral cavity bleeding compared to VKA continuation or interruption without bridging [OR=1.93, 95%CI 1.03-3.60]. After correction for age, sex, treatment intensity, indication, TTR percentage, treatment duration and use of antiplatelet drugs, perioperative bridging remained significantly associated with an increased non-oral cavity bleeding risk [OR=2.18, 95%CI 1.14-4.16].

A thromboembolic event within 30 days occurred in 5/2329 procedures (0.2%, 95%CI 0.1-0.5%). Of these thromboembolic events, three occurred after a low-risk (3/2004, 0.1%) and two after a high-risk procedure (2/325, 0.6%) [p=0.09]. Three occurred after VKA continuation (3/1383, 0.2%) and two after a procedure for which VKA therapy was

interrupted (2/946, 0.2%) [p=0.98]. Of the latter two events, one occurred in the non-bridging group (1/549, 0.2%) and the other in the bridging group (1/397, 0.3%) [p=0.82]. Hospitalisation within 30 days occurred in 100/2329 procedures (4.3%, 95%CI 3.5-5.2). Reasons for hospitalisation were: intracranial bleeding (1/100), ischaemic event (6/100), post-dental treatment haemorrhage (8/100), and 85/100 were unrelated to dental treatment or perioperative management.
A fatal event within 30 days occurred in 5/2329 procedures (0.2%, 95%CI 0.1-0.5). None of these were related to the dental procedure or management.

DISCUSSION

We evaluated the periprocedural management and clinical outcome after dental procedures in patients on VKA, in a real-world setting, and identified predictors for post-procedural oral cavity bleeding. Depending on the procedure risk, we observed an oral cavity bleeding rate of 3% after low-risk procedures and 6% after high-risk procedures. These rates are in accordance with previously reported bleeding rates (1,3,11). In contrast to international guidelines, the ACTA guideline incorporates the number of teeth involved in the procedure as a factor for bleeding risk. A previous study in 439 patients on VKA showed that for every extra extracted tooth the risk of bleeding increased by 28% (12). We also observed differences in bleeding rates after low-risk and high-risk procedures, which suggest that it is justifiable to categorise dental procedures accordingly. The specification of the number of teeth (1-3 low-risk, >3 high-risk) makes it easier for the dental practitioner and anticoagulation clinics to assess the bleeding risk of the procedure, which should be incorporated in decision making regarding periprocedural VKA management (5,7).
In our study, patient-related factors associated with an increased risk of bleeding were: increasing age (high-risk procedures) and concomitant exposure to antiplatelet therapy (low-risk procedures) or NSAIDs (high-risk procedures), which have also been reported in previous studies (1,13). Therapeutic quality control (e.g. lower TTR%) was not associated with an increased bleeding risk in our multivariate models, irrespective of the procedure risk, making it unlikely that TTR differences could explain the difference in bleeding risk between high-risk and low-risk procedures. Despite the well-known increased risk of bleeding associated with NSAID use (14), dental practitioners often prescribe these drugs for management of dental pain and swelling. Based on guideline recommendations (15) and our own findings, we discourage the use of NSAIDs for pain relief after invasive dental treatment, especially after high-risk procedures, in patients using VKA.

Considering periprocedural VKA management, like prior studies (16-18), our results indicate that VKA can be continued safely in low-risk procedures, in combination with a local pro-haemostatic agent, provided that the INR is at a therapeutic level before the procedure, since a supra-therapeutic INR before the procedure (INR>3.5) or absence of an objectified INR from the clinic within 72 h before the procedure, were independent predictors for bleeding. Furthermore, our data clearly indicates a risk reduction (approximately 50%) of bleeding when TXA mouthwash is prescribed during VKA continuation. The exact effect of TXA has been a point of discussion. Some studies (16,19), reported a lower bleeding rate when used after dental procedures, while another (20), found no differences in bleeding between exposure groups. Most of these studies were relatively small though, with heterogeneous periprocedural management and with very few bleeding complications, thus likely to be underpowered to find differences in outcomes between exposures if present.

In low risk-procedures, the risk of oral cavity bleeding was lower in patients who continued VKA treatment in combination with TXA compared to those bridged with LMWH, but similar compared to patients in whom VKA therapy was interrupted without bridging therapy. In one-third of the low-risk procedures, VKA therapy was temporarily interrupted where it should have been continued according to the guidelines. The most likely explanation for this finding is that anticoagulation clinics interrupt VKA therapy if deemed necessary by the dental practitioner. Assuming that the anticoagulation clinic will guard the thromboembolic safety of the patient, the dental practitioner often advocates an INR as low as possible before invasive treatment to prevent bleeding (21). On the other hand, anticoagulation clinics assume that the low INR is necessary to prevent bleeding and try to meet the request of clinicians by interrupting VKA therapy. On a population level, this causes heterogeneous VKA management, and in the end exposes a part of the patients to a higher bleeding risk if bridging therapy is initiated. Clear communication between dental practitioners and anticoagulation clinics is therefore required before deviating from management guidelines.

For high-risk procedures, we advise to interrupt VKA treatment and to avoid the use of NSAIDs as analgesics. The beneficial effect of TXA mouthwash was not statistically significant in high-risk procedures, likely due to a lack of power. We suggest that, in line with low-risk procedures, its use may lower the bleeding risk and should be considered regardless of management strategy.

Another important observation is that where VKA therapy was interrupted, bridging therapy was initiated by the anticoagulation clinic in a substantial proportion of both the low-risk (~1/3) and high-risk (~1/2) procedures. A previous study, evaluating both dental and other surgical procedures (n=222), showed that in daily practice adherence to bridging guidelines at anticoagulation clinics is suboptimal and LMWH therapy is

frequently initiated without a proper indication (22). Furthermore, these authors even concluded that the decision for bridging was often not based on the thromboembolic risk of the patient or the bleeding risk of the procedure, despite the increased bleeding risk associated with bridging (22). We also found an increased risk of clinically relevant oral and non-oral-bleeding associated with bridging therapy, and very low thromboembolic event rates, irrespective of the procedure risk or chosen management strategy. Since it has been shown that perioperative bridging therapy is associated with an increased risk of bleeding without lowering the risk of thromboembolic events (23-25), we advise that bridging should be kept to a minimum and only used in patients at the highest risk of thromboembolic complications during VKA interruption, such as recent stroke or venous thromboembolism, mechanical mitral valves and isolated atrial fibrillation with $CHA_2DS_2Vasc > 7$), in accordance with the Dutch guideline (26).
It is expected that direct oral anticoagulants will continue to increasingly replace VKA for the majority of patients requiring anticoagulation therapy. Due to their predictable pharmacokinetics, rapid onset of action, and short half-lives, these drugs can be interrupted for a shorter time than VKA and require no bridging with LMWH during interruption, irrespective of the patient's thrombotic risk (26). Although this simplifies perioperative management, dental surgeons and dentists should be aware of direct oral anticoagulants (DOAC) use by their patients and take adequate precautions to prevent bleeding in case of DOAC continuation.

Strengths and limitations

An important strength of our study is the large number of included dental procedures, which allowed us to compare clinical outcomes after different management strategies in both low and high bleeding risk procedures. The fact that these procedures were not performed in a trial setting enhances the generalizability of our findings. It is estimated that one in every six patients on chronic anticoagulant therapy is annually assessed for periprocedural VKA management for an elective procedure, which illustrates the importance of our findings beyond only dental procedures (6).
A few limitations of our study should be mentioned. First, we had no data on local dental influences that may affect the risk of oral cavity bleeding, such as the condition of the extracted teeth, the state of the surrounding gums, and local pro-haemostatic measures undertaken by the dentist or oral surgeon to prevent bleeding (e.g. proper sutures) (1). This impaired us to correct our models for these potential confounding factors. However, these factors are usually not communicated between dental practitioners and anticoagulation clinics, and it is safe to assume that dental practitioners always try to achieve primary haemostasis during treatment, making our results representative for daily practice.

To specifically relate these local factors to clinical outcome, in combination with the perioperative management strategy, a prospective study should be conducted in which both anticoagulation clinics, dentists, and oral surgeons provide the required information.

Second, given the retrospective study design and use of administrative data, we cannot definitively exclude the possibility of omission or misclassification of procedures and outcomes. We minimised the risk of misclassification by manually checking individual patient files independently by two investigators and by excluding procedures if these were inadequately described. In case of omission of bleeding events, it is unlikely that these bleedings are systemically related to a specific dental procedure or management strategy. Therefore we deem the chance of significant bias of our results due to the omission of bleeding events as low.

Conclusions

Most predictors of oral cavity bleeding were specifically related to periprocedural management and differed between low-risk and high-risk dental procedures, justifying different bleeding risk categories. Our observations emphasise the importance of adherence to VKA management guidelines, in which dental procedures should be categorised into low-risk and high-risk, each with specific perioperative management strategies. Overall, the concomitant use NSAIDs during dental treatment as analgesics should be avoided. VKAs can safely be continued in low-risk dental procedures in combination with tranexamic acid mouthwash provided that the INR≤3.5. In high-risk procedures, VKA should be interrupted and combined with tranexamic acid mouthwash. Bridging should only be applied in patients at highest risk of thromboembolic complications.

REFERENCES

1. Iwabuchi H, Imai Y, Asanami S, Shirakawa M, Yamane GY, Ogiuchi H, et al. Evaluation of postextraction bleeding incidence to compare patients receiving and not receiving warfarin therapy: a cross-sectional, multicentre, observational study. BMJ open. 2014;4(12):e005777-002014-005777.
2. Evans IL, Sayers MS, Gibbons AJ, Price G, Snooks H, Sugar AW. Can warfarin be continued during dental extraction? Results of a randomized controlled trial. Br J Oral Maxillofac Surg. 2002;40(3):248-52.
3. Hong C, Napenas JJ, Brennan M, Furney S, Lockhart P. Risk of postoperative bleeding after dental procedures in patients on warfarin: a retrospective study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(4):464-68.
4. Ramli R, Abdul Rahman R. Minor oral surgery in anticoagulated patients: local measures alone are sufficient for haemostasis. Singapore Dent J. 2005;27(1):13-16.
5. van Diermen DE, van der Waal I, Hoogstraten J. Management recommendations for invasive dental treatment in patients using oral antithrombotic medication, including novel oral anticoagulants. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(6):709-16.
6. Spyropoulos AC, Al-Badri A, Sherwood MW, Douketis JD. Periprocedural management of patients receiving a vitamin K antagonist or a direct oral anticoagulant requiring an elective procedure or surgery. J Thromb Haemost. 2016;14(5):875-85.
7. Douketis JD, Spyropoulos AC, Spencer FA, Mayr M, Jaffer AK, Eckman MH, et al. Perioperative management of antithrombotic therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e326S-350S.
8. Broekema FL, van Minnen B, Jansma J, Bos R. Risk of bleeding after dentoalveolar surgery in patients taking anticoagulants. Br J Oral Maxillofac Surg. 2014;52(3):e15-9.
9. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost. 1993;69(3):236-39.
10. Spyropoulos AC, Douketis JD, Gerotziafas G, Kaatz S, Ortel TL, Schulman S, et al. Periprocedural antithrombotic and bridging therapy: recommendations for standardized reporting in patients with arterial indications for chronic oral anticoagulant therapy. J Thromb Haemost. 2012;10(4):692-4.
11. Eichhorn W, Burkert J, Vorwig O, Blessmann M, Cachovan G, Zeuch J, et al. Bleeding incidence after oral surgery with continued oral anticoagulation. Clin Oral Investi. 2012;16(5):1371-6.
12. Febbo A, Cheng A, Stein B, Goss A, Sambrook P. Postoperative Bleeding Following Dental Extractions in Patients Anticoagulated With Warfarin. J Oral Maxillofac Surg. 2016;74(8):1518-23.
13. Kataoka T, Hoshi K, Ando T. Is the HAS-BLED score useful in predicting post-extraction bleeding in patients taking warfarin? A retrospective cohort study. BMJ Open. 2016;6(3):e010471.
14. Braganza A, Bissada N, Hatch C, Ficara A. The effect of non-steroidal anti-inflammatory drugs on bleeding during periodontal surgery. J Periodontol. 2005;76(7):1154-60.
15. van Diermen DE, Aartman IH, Baart JA, Hoogstraten J, van der Waal I. Dental management of patients using antithrombotic drugs: critical appraisal of existing guidelines. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(5):616-24.
16. Ramstrom G, Sindet-Pedersen S, Hall G, Blomback M, Alander U. Prevention of postsurgical bleeding in oral surgery using tranexamic acid without dose modification of oral anticoagulants. J Oral Maxillofac Surg. 1993;51(11):1211-6.
17. Al-Mubarak S, Al-Ali N, Abou-Rass M, Al-Sohail A, Robert A, Al-Zoman K, et al. Evaluation of dental extractions, suturing and INR on postoperative bleeding of patients maintained on oral anticoagulant therapy. Br Dent J. 2007;203(7):E15;discussion 410-1.

18. Bacci C, Maglione M, Favero L, Perini A, Di Lenarda R, Berengo M, et al. Management of dental extraction in patients undergoing anticoagulant treatment. Results from a large, multicentre, prospective, case-control study. Thromb Haemost. 2010;104(5):972-5.
19. Sindet-Pedersen S, Ramstrom G, Bernvil S, Blomback M. Hemostatic effect of tranexamic acid mouthwash in anticoagulant-treated patients undergoing oral surgery. N Engl J Med. 1989;320(13):840-3.
20. Soares EC, Costa FW, Bezerra TP, Noqueira CB, de Barros Silva PG, Batista SH. Postoperative hemostatic efficacy of gauze soaked in tranexamic acid, fibrin sponge, and dry gauze compression following dental extractions in anticoagulated patients with cardiovascular disease: a prospective, randomized study. Oral Maxillofac Surg. 2015;19(2):209-16.
21. van Diermen DE, Bruers JJ, Hoogstraten J, Bovenlander M, van den Bosch A, van der Waal I. Treating dental patients who use antithrombotic medication: a survey of dentists in the Netherlands. J Am Dent Assoc. 2011;142(12):1376-82.
22. Eijgenraam P, ten Cate H, ten Cate-Hoek AJ. Practice of bridging anticoagulation: guideline adherence and risk factors for bleeding. Neth J Med. 2014;72(3):157-64.
23. Siegal D, Yudin J, Kaatz S, Douketis JD, Lim W, Spyropoulos AC. Periprocedural heparin bridging in patients receiving vitamin K antagonists: systematic review and meta-analysis of bleeding and thromboembolic rates. Circulation. 2012;126(13):1630-9.
24. Clark NP, Witt DM, Davies LE, Saito EM, McCool KH, Douketis JD, et al. Bleeding, Recurrent Venous Thromboembolism, and Mortality Risks During Warfarin Interruption for Invasive Procedures. JAMA Intern Med. 2015;175(7):1163-8.
25. Douketis JD, Spyropoulos AC, Kaatz S, Beckers RC, Caprini JA, Dunn AS, et al. Perioperative Bridging Anticoagulation in Patients with Atrial Fibrillation. N Engl J Med. 2015;373(9):823-33.
26. Dutch Society for Internal Medicine: Guideline antithrombotic therapy.
27. http://richtlijnendatabase.nl/richtlijn/antitrombotisch_beleid/perioperatief_beleid_bij_antistolling.html. Accessed August 15, 2016.

CHAPTER 9

Can we prevent venous thrombosis with statins: an epidemiologic review into mechanism and clinical utility

Expert Rev Hematol 2016;9:1023-30

Willem M. Lijfering[1], Joseph S. Biedermann[2], Marieke J.H.A. Kruip[2], Frank W.G. Leebeek[2], Frits R. Rosendaal[1], Suzanne C. Cannegieter[1,3]

[1] Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands
[2] Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands
[3] Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, The Netherlands

ABSTRACT

Introduction

Statins may be causally associated with a decreased risk of venous thrombosis. If so, this could be a substantive breakthrough since statins do not increase the risk of bleeding and could therefore be used as a safer antithrombotic drug. However, scepticism exists on the observed reduction of venous thrombosis by statins, as it may have been confounded by healthy user effects or other biases.

Areas covered

The main focus of this review will be the biases that may have arisen in clinical studies that investigated the relationship between statin use and risk of venous thrombosis. We also discuss the suggested causal association from a pathophysiological perspective. Furthermore, we integrate the knowledge from clinical and pathophysiological studies into a proposal for new study designs that are needed to sufficiently answer the question whether we can, and should, prevent recurrent venous thrombosis with statins.

Expert Commentary

A drug to prevent recurrent venous thrombosis in patients at risk of bleeding that does not induce bleeding and in which the number needed to treat for the prevention of venous thrombosis is sufficiently high, is a remedy that we should continue to look for, and for which statin therapy might be a suitable candidate.

INTRODUCTION

Venous thrombosis (deep vein thrombosis or pulmonary embolism) is a common and potentially lethal disease that occurs each year in about 1-2/1000 people (1). The condition can be prevented and treated with anticoagulants, but as a side effect bleeding often occurs (2). Currently, the duration of treatment of venous thrombosis with anticoagulants depends on whether the event was provoked or not (3). Most provoking risk factors, such as surgery, immobilization, and use of oral contraceptives, are of a transient nature. Presence of such a risk factor temporarily increases the 'thrombotic potential' of an individual, and hence the risk decreases once the risk factor is gone. This explains, for example, why recurrence risk is low (<1%/year) in patients who developed their first event after surgery (4). Patients with provoking risk factors are usually treated with anticoagulants for 3-6 months only, while patients with unprovoked thrombosis are prescribed anticoagulant treatment for a longer period (3). This extended treatment should be seen as prevention of a recurrence, which decision is based on its high incidence in patients with unprovoked events (30% within 5 years after the 3-6 months of oral anticoagulation) (5). Only 40-50% of all thrombosis patients can be classified as patients a first provoked event, which leads to a dilemma in the other 50-60%: discontinuing treatment may lead to a new thrombotic event, while continuing oral anticoagulant treatment is accompanied with a yearly 1-3% risk of major bleeding (2,3). Therefore, novel therapeutic strategies to prevent venous thrombosis that are not associated with bleeding complications are urgently needed.

In this review, we will discuss whether statins are causally associated with a decreased risk of venous thrombosis. If true, this could be a substantive breakthrough since statins are known not to cause bleeding. We will summarize clinical research that studied whether statins exert beneficial effects in preventing venous thrombosis. Next, we will discuss possible mechanisms from a pathophysiological perspective. Finally, we will integrate the knowledge obtained from clinical- and pathophysiological studies into a proposal that is needed to sufficiently answer the question if we can prevent recurrent venous thrombosis with statin therapy.

CLINICAL STUDIES

Initial findings

In 2000, Grady and colleagues were the first to report that statin use was associated with a 50% reduced risk for development of venous thrombosis in post-menopausal women starting estrogen and progestin therapy (6). Since then, many other studies have been published on the association between statin use and decreased risk of venous thrombosis. For instance, a meta-analysis of seven observational studies revealed that statin use was associated with a significantly lower risk of VTE compared to non-statin use (odds ratio 0.62, 95% confidence interval (CI), 0.45-0.86) (7). However, statin use is associated with several preventive effects in observational studies: it is not only associated with lower risks of venous thrombosis, but also with lower risks of arrhythmia, multiple sclerosis, Alzheimer's dementia, infections, AIDS, cancer mortality, and even motor vehicle accidents (8-10). Because these effects do not seem to be due to lower lipid levels, this raises the suspicion that at least some of the observed associations are due to non-causal mechanisms, such as bias.

Bias

Bias can be defined as a process at any stage of causal inference which tends to produce results or conclusions that differ systematically from the truth. The landmark article by Sacket in 1979, listed 35 biases, of which we will discuss 3 types that could explain the observation that statins are associated with a decreased risk of venous thrombosis (11). The first lies in what has been called the "healthy user" effect, i.e. that statins are prescribed preferentially to individuals with a favourable risk profile or that the healthiest users are analysed in some observational studies (12). However, for venous thrombosis it is unlikely that this healthy user effect fully explains the positive association with statin use, as (high risk of) arterial cardiovascular disease is an indication for statin use, of which some risk factors (age, male sex, obesity, smoking) are shared risk factors for both conditions (13). Thus, participants in observational studies who use statins should have a less favorable cardiovascular risk factor profile than non-users and are therefore at *higher* (and not lower) baseline risk for venous thrombosis.

However, other types of bias may have contributed to the observed lower risk of venous thrombosis while using a statin, for instance in studies that included individuals who had been using statins for some time prior to study entry (7). Such "prevalent users" can introduce two types of bias: 1) underascertainment of events that occur early after starting treatment (survivor bias) and 2) the inability to control for those who do or do not adhere to statin treatment (adherence bias) (14,15).

In terms of survivor bias, for statins there is indirect evidence that the risk for venous

thrombosis is increased in the first months of statin treatment, since an indication for statin therapy is a recently experienced arterial cardiovascular event. Because it has been reported that patients with acute arterial cardiovascular disease are at increased risk of subsequent venous thrombosis and death for a short time period, underascertainment of venous thrombotic events can result from early attrition of patients on statins who are most susceptible but may have died or be too sick to be enrolled in an observational study (16,17).

In terms of adherence bias, prevalent users in a study, by definition, use a statin at time of inclusion, while those who had an indication for statin treatment yet failed to continue with their treatment are abraded as non-statin users. Adherence to a drug is a marker for a constellation of unmeasured factors and likely associated with better outcome, independent of the drug use itself. This is true for all drugs, including statin use and even for placebo use. For instance, several randomized controlled clinical trials in which patients who were adherent to placebo showed 30–60 percent reduced risks of death from cardiovascular disease, as compared with non-adherent placebo users, which magnitude of the association was not materially affected by adjustment for several potential confounders (18-20).

For these two reasons, it is important to take prevalent users into account when studying effects of statin treatment on the risk of venous thrombosis in observational studies. For this, one can use a so-called 'new-user design' (14). Such a design begins by identifying all individuals in a predefined population (both in terms of people and time) who for the first time start a course of treatment with a statin. Study follow-up for endpoints begins at precisely the same time as initiation of statin therapy or t=0. Data for all patient characteristics are obtained at a time just before t=0. Observational studies can be performed by initially assembling a cohort consisting of only new users and an appropriate comparison group or by identifying new users and the comparison group from an existing cohort. This definition is similar to the way in which data are analyzed in a clinical trial, where t=0 is the time of randomization (usually just before treatment begins), except of course that treatment is not assigned by randomization. A new-user design differs from most observational studies in that it excludes prevalent users. For this matter it is interesting to note that in aforementioned meta-analysis, only one of the seven mentioned studies included statin initiators in their study (new-user design) and reported an odds ratio of 1.02 (95% CI, 0.88-1.18), which is in contrast to the overall odds ratio of 0.62 (95% CI, 0.45-0.86) (7,9,21). This raises the suspicion that the observed association between statin use and a decreased risk of venous thrombosis suffered from prevalent users in these studies leading to bias. However, other studies in which prevalent user bias was excluded by design have been published, including results from a trial and a meta-analysis of trials, which we will discuss below (22,23).

Class effect?

Side effects of drugs are not necessarily class effects, particularly when the mechanism of the side effect differs from the primary mechanism of the drug. It is known that the mechanism of statins varies between the types of statins that are currently on the market today, showing different reducing effects on LDL, atherosclerosis and inflammation. This reduction is the least strong in pravastatin users, followed by simvastatin users and atorvastatin users and is strongest in rosuvastatin users (24,25). There are some studies that suggest that dyslipidemia, inflammation or atherosclerosis, i.e. determinants for arterial cardiovascular disease, also increase the risk of venous thrombosis (26-28). Therefore, an analysis done by type of statin to venous thrombosis risk seems sensible as, in case of a causal association mediated through dyslipidemia, inflammation or atherosclerosis, the effect on venous thrombosis risk should be strongest in rosuvastatin and weakest in pravastatin users. As summarized in Table 1, in a cohort study of nearly 2 million individuals from the United Kingdom, in which a new-user design was used, authors showed that rosuvastatin use was associated with the strongest (approximately 40%) reduced risk of venous thrombosis (29). These results closely resemble the results from randomized controlled trials (22,23). For the occurrence of venous thrombosis, a pre-defined analysis of a randomized clinical trial in which apparently healthy individuals were randomized to rosuvastatin or placebo (JUPITER trial) showed a 40% risk reduction when using rosuvastatin as compared with placebo (Table 1) (22). In the absence of other randomized trials with venous thrombosis as the primary endpoint, Rahimi and colleagues presented a pooled analysis of 29 randomized statin studies in which venous thrombotic events were reported as serious adverse events (23). They failed to confirm a risk reduction of venous thrombosis by statin treatment. However, authors found that individuals who were randomized to rosuvastatin still had an approximately 40% reduced risk of venous thrombosis (hazard

Table 1. Effect of statin therapy on venous thrombosis by type of statin

	Observational study [31] Hazard ratio (95% CI)		**Clinical trials [24,25] Hazard ratio (95% CI)**	
	Women	**Men**	**Jupiter trial**	**Rahimi et al***
No statin	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Pravastatin	1.05 (0.80-1.38)	1.00 (0.76-1.31)		1.17 (0.81-1.69)
Simvastatin	0.91 (0.83-0.99)	0.88 (0.80-0.97)		0.94 (0.71-1.25)
Atorvastatin	0.86 (0.76-0.99)	0.86 (0.75-0.97)		0.82 (0.56-1.19)
Rosuvastatin	0.61 (0.36-1.03)	0.53 (0.29-0.95)	0.57 (0.37-0.86)	0.65 (0.33-1.28)
Any statin	Unavailable	Unavailable		0.93 (0.82-1.07)

* Excluding results from the Jupiter trial

ratio 0.65; 95CI, 0.33-1.28) (Table 1). Albeit confidence intervals were wide in the study from Rahimi et al, results from Table 1 suggest a dose response relation where the statin that is most related with halting/regression of atherosclerosis, dyslipidemia and inflammation (i.e. rosuvastatin) also provides the largest risk reductions for the occurrence of venous thrombosis.

PATHOPHYSIOLOGY

Statins and (early) atherosclerosis

In 2003, the hypothesis was sparked that atherosclerosis leads to venous thrombosis (30). As in this study atherosclerosis measurements were performed after venous thrombosis occurred, its temporal relation and causality were not entirely clear (31). Currently, there is little evidence available that venous thrombosis and atherosclerosis are causally associated (13). However, there is some biological evidence that may give credence to the causality of this association since the hemostatic system seems to be able to accelerate atherosclerosis (31). This was demonstrated in mouse studies with hypercoagulable and diminished coagulation phenotypes on an atherosclerotic background, where diminished coagulation provided protection against atherosclerosis development, whereas hypercoagulable mice developed more severe atherosclerosis (32). In human histological studies, it has been shown that a procoagulant state is more abundantly present in early-stage atherosclerotic lesions than in advanced atherosclerotic lesions (33-35). Why coagulation factors are more abundantly present within *early* atherosclerotic vessels than in *advanced* atherosclerosis, is as yet unknown, but may be attributable to primary protective mechanisms against vascular injury (36). With the advent of *in vivo* carotid MRI screening, one can now distinguish early from advanced atherosclerosis (37,38). It is therefore possible to perform clinical studies to quantify as to whether both early and/or advanced atherosclerosis increases the risk of venous thrombosis. Such studies could clarify why of all the statins that are currently available, the most potent anti-atherosclerotic ones, are associated with a lower risk of venous thrombosis (22,23,29). As far as we know such studies have not been conducted yet.

Statins and dyslipidemia

As lipid levels can be modulated by lifestyle intervention and statin therapy, the potential association between lipids and venous thrombosis and its underlying pathophysiology is a relevant issue worth pursuing (39). However, whether lipid levels themselves are associated with venous thrombosis is controversial due to different

results among epidemiological studies. A previous meta-analysis demonstrated that mean levels of total cholesterol were higher and HDL cholesterol levels were lower in venous thrombosis patients than in controls (26). However, the majority of the reports on lipids and venous thrombosis in this meta-analysis were small case-control studies, and individually controlling for several confounders was not possible (26). Moreover, there was severe heterogeneity between studies meaning that there was a large variation in study outcomes between studies, of which the larger ones found no association with lipid levels and venous thrombosis. Several large population-based cohort studies have been published since the aforementioned meta-analysis, and these additional studies found no association between dyslipidemia and venous thrombosis after controlling for confounding factors and competing risk (40-42). In addition, non-statin lipid lowering drugs (i.e. fibrates) are not associated with a reduced venous thrombosis risk (43). Therefore, we consider it unlikely that statins decrease venous thrombosis risk by their lipid lowering activities.

Statins and inflammation

Several lines of evidence, ranging from experimental models to population-based studies, support the notion that inflammation is a driver of atherosclerosis (44). From an epidemiological perspective, numerous studies have shown that the inflammatory marker hs-CRP is not only associated with atherosclerosis, but also with an increased risk of venous thrombosis, and with higher levels of procoagulant factor VIII (45-47). However, a Mendelian randomization study convincingly showed that hs-CRP levels are not a cause of venous thrombosis (47). Still, statins, initially manufactured to target dyslipidemia and slow down atherosclerosis, showed that they also have anti-inflammatory properties (48). Since atherosclerosis can produce both an inflammatory and procoagulant response, reduction of inflammation by statins could be a driving force behind the reduced venous thrombosis risk that has been observed in statin users (31,44). Interestingly, one study of 26 patients who had venous thrombosis found that a 3-day administration of atorvastatin reduced inflammation as evidenced by reduced interleukin (IL) IL-6, IL-8, and soluble P-selectin, together with increased anti-inflammatory IL-10, without any significant effect on hs-CRP (49). Because of the short time interval this study suggests potential benefits from statin administration with regard to reduced venous thrombosis risk that is in part driven by an immediate anti-inflammatory (i.e. not related with atherosclerosis) effect.

Statins and platelet activation

Another mechanism through which statins may decrease the risk of venous thrombosis is by inhibiting platelet activation and consequently aggregation (50). Animal models

have shown that platelet activation plays a key role in the initiation of thrombus formation in deep vein thrombosis (51,52). Furthermore, *in vitro* studies indicate that statins inhibit platelet activation via several lipid-independent mechanisms including the inhibition of thromboxane A2 (TxA2) formation (53). Since enhanced platelet aggregation has been reported in patients with venous thrombosis, we recently decided to investigate if there is an effect of rosuvastatin on TxA2-mediated platelet activation in individuals with a history of venous thrombosis in the START trial (54,55). We randomized 25 individuals to rosuvastatin 20 mg daily for 28 days and 25 individuals to no statin, and observed no effect of TxA2-mediated platelet activation in rosuvastatin users. These findings show that it is unlikely that the association of a decreased risk of venous thrombosis in rosuvastatin users is explained by decreased TxA2-mediated platelet activation. However, a limitation of this study was that only one assay was used to evaluate platelet function (56). Therefore, we cannot exclude potential other antiplatelet effects of rosuvastatin as can be measured using different platelet function tests.

Statins and coagulation

Colli *et al* were the first in 1997 to report that statins interfere with activation of the clotting system and the coagulation cascade (57). In that study, authors observed that tissue factor was suppressed in macrophages that were incubated for 20-24 hours with simvastatin. Many reports followed, for example by Undas and colleagues who showed that once daily use of simvastatin 40 mg given for 3 days in 14 healthy volunteers resulted in a reduction of thrombin formation, that was of a similar magnitude to that observed after 90 days of statin therapy (58-61). Another study found evidence for rapid alterations in fibrin clot structure/ function induced by statins in venous thrombosis patients treated with atorvastatin 40 mg once daily for 3 days (62). Based on these reports, the authors suggested that statin use may decrease the risk of venous thrombosis by downsizing coagulation activation. However, these findings should be interpreted with caution as not all studies consistently reported a favourable outcome of statin treatment on coagulation factor levels. For example, in a randomized study from Dangas *et al* (n=93), an increase in fibrinogen level was observed when individuals were exposed to pravastatin compared with placebo (63). Recently published findings from randomized controlled trials of statin therapy suggest some effect of lowering levels of von Willebrand factor and D-dimer (64,65). However, funnel plot analyses showed that these positive results might be due to publication bias. Furthermore, only one trial (n=60) reported on the potential effects of rosuvastatin to the hemostatic system (66). Observational studies that found a possible relation between statin use and a decreased level of procoagulant factors, could have been hampered by methodological issues such as survivor bias and adherence bias as they

Table 2. Median levels of procoagulant factors in fibrate and statin users in control subjects of the MEGA study

		Statin users				
Median level (IQR)	**Fibrate users** (n=7)	**Fluvastatin** (n=10)	**Simvastatin** (n=144)	**Atorvastatin** (n=136)	**Pravastatin** (n=53)	**Rosuvastatin** (n=11)
Fibrinogen, g/L	3.3 (2.5-4.0)	4.0 (3.3-4.3)	3.5 (3.1-4.0)	3.6 (3.1-4.1)	3.5 (3.2-4.1)	3.6 (3.1-4.0)
Factor II,† IU/dL	111 (103-122)	109 (97-127)	113 (104-122)	110 (101-118)	114 (133-127)	102 (88-124)
Factor VII,† IU/dL	139 (110-152)	121 (99-134)	118 (98-138)	124 (107-139)	127 (118-153)	97 (78-129)
Factor VIII:Ag, IU/dL	123 (70-204)	133 (109-205)	130 (106-159)	114 (92-154)	119 (84-145)	101 (86-137)
VWF, IU/dL	111 (58-170)	115 (91-126)	118 (96-159)	111 (87-150)	103(71-144)	95 (85-137)
Factor IX,† IU/dL	114 (103-159)	118 (99-142)	111 (101-125)	109 (100-124)	112 (98-125)	101 (92-109)
Factor X,† IU/dL	118 (102-146)	111 (103-132)	119 (104-130)	120 (110-133)	120 (100-133)	111 (98-125)
Factor XI, IU/dL	115 (110-156)	101 (86-131)	105 (90-121)	104 (94-117)	104 (88-110)	93 (86-122)

† Control subjects on vitamin K antagonists excluded. IQR denotes inter quartile range.

included prevalent users (14,67). In addition, none of these studies may be generalizable to venous thrombosis patients as they have generally been conducted in patients with hyperlipidemia, diabetes or other disease states unrelated with venous thrombosis.
In an observational study with an active-comparator design, the drug of interest is compared with another agent commonly used for the same indication, rather than with no treatment (a 'non-user' group) (68). This principle helps to ensure that treatment groups have similar treatment indications, attenuating both measured and unmeasured differences in patient characteristics. Studies that used such a design on the issue as to whether coagulation factor levels are influenced by statins, have as far as we know, not been reported previously. For the current review, we explored such a study-design in the control individuals of the MEGA study of which results are shown in Table 2. We analyzed if there was a difference in the median level of several coagulation factors in individuals who were treated with lipid lowering drugs, i.e. either statins or fibrate, at time of blood sampling. Although from Table 2 it appears that of the lipid lowering therapies that were prescribed, statin users had a less hypercoagulable profile than fibrate users (with rosuvastatin users having the most favourable coagulation profile), we believe that this finding should be interpreted with caution as numbers are small.
A powerful design not suffering from any of the aforementioned limitations to answer the question whether coagulation factors are influenced by statin therapy is a sufficiently powered randomized trial in which the primary outcome is change in coagulation factor level in patients with prior venous thrombosis. We are currently conducting such a trial (START study; www.clinicaltrials.gov NCT01613794) in which patients, after having received treatment with anticoagulants for venous thrombosis, are randomized to rosuvastatin 20mg once daily for 1 month to study the potential anticoagulant properties of statins. The study is powered on coagulation factor VIII, as a high factor VIII level is well associated with both first and recurrent venous thrombosis (69-71). Of note, START will only look at the immediate (1 month) effect of rosuvastatin treatment to coagulation factor levels. Randomized studies that take longer time effects of rosuvastatin therapy to coagulation factor levels into account may be covered by the SAVER trial (www.clinicaltrials.gov NCT02679664).

EXPERT COMMENTARY

Whether venous thrombosis can be prevented with the use of statins is questionable as the available data are scant, controversial and likely biased, with few replicated studies. Nevertheless, pathophysiological insights reveal a potential mechanism through anticoagulant and/or or anti-inflammatory properties of statins, most notably

rosuvastatin, possibly by targeting (early) atherosclerosis. That cheap and accessible anti-atherosclerotic drugs, like statins, might prevent venous thrombosis without inducing bleeding, offers currently not enough ground to start statin treatment in these patients. For this we need more and better evidence.

FIVE YEAR VIEW

The following research questions and accompanying designs are essential to answer the question if statins can prevent venous thrombosis.

1. We need to know the pharmacological mechanism of *how* statins are able to decrease the risk of venous thrombosis. This should be studied in sufficiently powered randomized clinical trials or in active comparator study designs that are externally validated and primarily set up for this reason. We are currently conducting the START trial (www.clinicaltrials.gov NCT01613794) to analyze the immediate 1-month effects on coagulation of 20 mg rosuvastatin use once daily in 250 randomized patients with prior venous thrombosis. Since rosuvastatin is the statin that consistently showed the strongest association between its intake and a reduced risk of venous thrombosis, we will consider a negative signal from this trial as negative for the whole class of statins (22,23,29). A possible positive signal may not rule in potential beneficial effects on the coagulation profile from the other statins that are currently on the market. Of note, START can only look at the immediate (1 month) effect of rosuvastatin treatment to coagulation factor levels.
2. We need to know if various stages of atherosclerosis can increase the risk of venous thrombosis. If so, the culprit behind the supposed causal association between statin intake and reduced coagulation could be early atherosclerosis. Of the latter, previous research has shown that increased coagulation activity is specifically observed in early atherosclerotic lesions, but we do not know if early atherosclerosis increases venous thrombosis risk (31,33). To study this, we need to perform a clinical study (either case-control or follow-up) in which early and advanced stages of atherosclerosis are viewed accurately *in vivo*, which is now possible with non-invasive high resolution MRI (37,38). External validation of results from a previous autopsy study, is needed to endorse that early atherosclerosis is also associated with an *ex-vivo* prothrombotic state/venous thrombosis as compared with no atherosclerosis or advanced atherosclerosis, with rigorous adjustments for potential confounding factors (33).

3. If the hypotheses in abovementioned study proposals turn out to be true, this will not immediately change clinical practice as for this a randomized trial with clinical endpoints is needed. Large randomized trials are not started in a vacuum, but require a high prior probability of success, some insight in pathophysiological mechanisms, and need to be performed in specific groups who may benefit the most. The results of the studies as proposed in 1) and 2) will provide sufficient evidence to determine if such a trial should be conducted. Since the question as to whether a therapy is effective is answered by the number needed to treat per year (NNT per year), the secondary prevention of venous thrombosis after an unprovoked event with statins, where reasonable NNTs may be plausible because of high recurrent venous thrombosis event rates, could be feasible (72-75). It is in this aspect interesting to note that the current ACCP guideline suggests to discontinue anticoagulant treatment in patients with venous thrombosis who are considered to be at high risk of anticoagulation related bleeding (3). Although statins are unlikely to be as effective as anticoagulant drugs, they do have the major advantage over anticoagulants that they do not induce bleeding. Therefore, a drug to prevent recurrent venous thrombosis in patients at risk of bleeding that does not induce bleeding and in which the NNT for the prevention of recurrence is sufficiently high, is a remedy that we should continue to look for, and for which statin therapy might be a suitable candidate.

REFERENCES

1. Naess IA, Christiansen SC, Romundstad P, et al. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost. 2007;5:692-9.
2. Veeger NJ, Piersma-Wichers M, Tijssen JG, et al. Individual time within target range in patients treated with vitamin K antagonists: main determinant of quality of anticoagulation and predictor of clinical outcome. A retrospective study of 2300 consecutive patients with venous thromboembolism. Brit J Haematol. 2005;128:513-9.
3. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest. 2016;149(2):315-52. 4. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353:1167-73.
4. Baglin T, Luddington R, Brown K, et al. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet. 2003;362:523-6.
5. Prandoni P, Noventa F, Ghirarduzzi A, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica. 2007;92:199-205.
6. Grady D, Wenger NK, Herrington D, et al. Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The Heart and Estrogen/progestin Replacement Study. Ann Intern Med. 2000;132:689-96.
7. Pai M, Evans NS, Shah SJ, et al. Statins in the prevention of venous thromboembolism: a meta-analysis of observational studies. Thromb Res. 2011;128:422-30.
8. Nielsen SF, Nordestgaard BG, Bojesen SE. Statin use and reduced cancer-related mortality. N Engl J Med. 2012;367:1792-802.
9. Dormuth CR, Patrick AR, Shrank WH, et al. Statin adherence and risk of accidents: a cautionary tale. Circulation. 2009;119:2051-7.
10. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? PLoS Med. 2012;9:e1001311.
11. Sackett DL. Bias in analytic research. J Chronic Dis. 1979;32:51-63.
12. Thomsen RW. The lesser known effects of statins: benefits on infectious outcomes may be explained by "healthy user" effect. BMJ. 2006;333:980-1.
13. Lijfering WM, Flinterman LE, Vandenbroucke JP, et al. Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. Semin Thromb Hemost. 2011;37:885-96.
14. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. Am J Epidemiol. 2003 Nov 1;158:915-20.
15. Danaei G, Tavakkoli M, Hernán MA. Bias in observational studies of prevalent users: lessons for comparative effectiveness research from a meta-analysis of statins. Am J Epidemiol. 2012;175:250-62.
16. Collins R, MacMahon S, Flather M, et al. Clinical effects of anticoagulant therapy in suspected acute myocardial infarction: systematic overview of randomised trials. BMJ. 1996;313:652-9.
17. Sørensen HT, Horvath-Puho E, Søgaard KK, et al. Arterial cardiovascular events, statins, low-dose aspirin and subsequent risk of venous thromboembolism: a population-based case-control study. J Thromb Haemost. 2009;7:521-8.
18. Influence of adherence to treatment and response of cholesterol on mortality in the coronary drug project. N Engl J Med 1980;303:1038–41.
19. Horwitz RI, Viscoli CM, Berkman L, et al. Treatment adherence and risk of death after a myocardial infarction. Lancet 1990;336:542–5.
20. Gallagher EJ, Viscoli CM, Horwitz RI. The relationship of treatment adherence to the risk of death after myocardial infarction in women. JAMA 1993;270:742–4.

21. Smeeth L, Douglas I, Hall AJ, et al. Effect of statins on a wide range of health outcomes: a cohort study validated by comparison with randomized trials. Br J Clin Pharmacol. 2009;67:99-109.
22. Glynn RJ, Danielson E, Fonseca FA, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360:1851-61.
23. Rahimi K, Bhala N, Kamphuisen P, et al. Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. PLoS Med. 2012;9:e1001310.
24. Nissen SE, Nicholls SJ, Sipahi I, et al. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA. 2006;295:1556-65.
25. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ. 2003;326:1423.
26. Ageno W, Becattini C, Brighton T, et al. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. Circulation. 2008;117:93-102.
27. Horvei LD, Grimnes G, Hindberg K, et al. C-reactive protein, obesity, and risk of arterial and venous thrombosis. J Thromb Haemost. 2016;14:1561-71.
28. Prandoni P, Bilora F, Marchiori A, et al. An association between atherosclerosis and venous thrombosis. N Engl J Med. 2003;348:1435-41.
29. Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. BMJ. 2010;340:c2197.
30. Prandoni P. Venous thromboembolism and atherosclerosis: is there a link? J Thromb Haemost. 2007;5:270-275.
31. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. N Engl J Med. 2011;364:1746-60.
32. Borissoff JI, Otten JJ, Heeneman S, et al. Genetic and pharmacological modifications of thrombin formation in apolipoprotein e-deficient mice determine atherosclerosis severity and atherothrombosis onset in a neutrophil-dependent manner. PLoS One. 2013;8:e55784.
33. Borissoff JI, Heeneman S, Kilinç E, et al. Early atherosclerosis exhibits an enhanced procoagulant state. Circulation. 2010;122:821-30.
34. Seehaus S, Shahzad K, Kashif M, et al. Hypercoagulability inhibits monocyte transendothelial migration through protease-activated receptor-1-, phospholipase-Cbeta-, phosphoinositide 3-kinase-, and nitric oxide-dependent signaling in monocytes and promotes plaque stability. Circulation. 2009;120:774-84.
35. With Notø AT, Mathiesen EB, Østerud B, et al. Increased thrombin generation in persons with echogenic carotid plaques. Thromb Haemost. 2008;99:602-8.
36. Kalz J, ten Cate H, Spronk HM. Thrombin generation and atherosclerosis. J Thromb Thrombolysis. 2014;37:45-55.
37. Cai JM, Hatsukami TS, Ferguson MS, et al. Classification of human carotid atherosclerotic lesions with in vivo multicontrast magnetic resonance imaging. Circulation. 2002;106:1368-73.
38. Zhang Y, Guallar E, Qiao Y, et al. Is carotid intima-media thickness as predictive as other noninvasive techniques for the detection of coronary artery disease? Arterioscler Thromb Vasc Biol. 2014;34:1341-5.
39. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143-421.
40. Holst AG, Jensen G, Prescott E. Risk factors for venous thromboembolism: results from the Copenhagen City Heart Study. Circulation. 2010;121:1896-903.
41. Brækkan SK, Hald EM, Mathiesen EB, et al. Competing risk of atherosclerotic risk factors for arterial and venous thrombosis in a general population: the Tromso study. Arterioscler Thromb Vasc Biol. 2012;32:487-91.

42. van Schouwenburg IM, Mahmoodi BK, Gansevoort RT, et al. Lipid levels do not influence the risk of venous thromboembolism. Results of a population-based cohort study. Thromb Haemost. 2012;108:923-9.
43. Ramcharan AS, Van Stralen KJ, Snoep JD, et al. HMG-CoA reductase inhibitors, other lipid-lowering medication, antiplatelet therapy, and the risk of venous thrombosis. J Thromb Haemost. 2009;7:514-20.
44. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340:115-26.
45. Folsom AR, Lutsey PL, Astor BC, et al. C-reactive protein and venous thromboembolism. A prospective investigation in the ARIC cohort. Thromb Haemost. 2009;102:615-9.
46. Quist-Paulsen P, Naess IA, Cannegieter SC, et al. Arterial cardiovascular risk factors and venous thrombosis: results from a population-based, prospective study (the HUNT 2). Haematologica. 2010;95:119-25.
47. Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein and risk of venous thromboembolism in the general population. Arterioscler Thromb Vasc Biol. 2010;30:1672-8.
48. Ridker PM, Rifai N, Pfeffer MA, et al. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 1999;100:230-5.
49. Zolcinski M, Cieśla-Dul M, Potaczek DP, et al. Atorvastatin favourably modulates proinflammatory cytokine profile in patients following deep vein thrombosis. Thromb Res. 2013;132:e31-5.
50. Puccetti L, Santilli F, Pasqui AL, et al. Effects of atorvastatin and rosuvastatin on thromboxane-dependent platelet activation and oxidative stress in hypercholesterolemia. Atherosclerosis. 2011;214:122–128.
51. Brill A, Fuchs TA, Chauhan AK, et al. von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. Blood. 2011;117:1400-710.
52. Joglekar MV, Ware J, Xu J, et al. Platelets, glycoprotein Ib-IX, and von Willebrand factor are required for FeCl(3)-induced occlusive thrombus formation in the inferior vena cava of mice. Platelets. 2013;24:205-12.
53. Pignatelli P, Carnevale R, Pastori D, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. Circulation. 2012;126:92–103.
54. Weber M, Gerdsen F, Gutensohn K, et al. Enhanced platelet aggregation with TRAP-6 and collagen in platelet aggregometry in patients with venous thromboembolism. Thromb Res. 2002;107:325-8.
55. Biedermann JS, Cannegieter SC, Roest M, et al. Platelet reactivity in patients with venous thrombosis who use rosuvastatin: a randomized controlled clinical trial. J Thromb Haemost. 2016;14:1404-9.
56. Israels SJ. Laboratory testing for platelet function disorders. Int J Lab Hematol. 2015;37:18-24.
57. Colli S, Eligini S, Lalli M, et al. Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. Arterioscler Thromb Vasc Biol. 1997;17:265-72.
58. Violi F, Calvieri C, Ferro D, et al. Statins as antithrombotic drugs. Circulation. 2013;127:251-7.
59. Undas A, Brummel-Ziedins KE, Mann KG. Anticoagulant effects of statins and their clinical implications. Thromb Haemost. 2014;111:392-400.
60. Undas A, Celinska-Löwenhoff M, Brummel-Ziedins KE, et al. Simvastatin given for 3 days can inhibit thrombin generation and activation of factor V and enhance factor Va inactivation in hypercholesterolemic patients. Arterioscler Thromb Vasc Biol. 2005;25:1524-5.
61. Undas A, Brummel KE, Musial J, et al. Simvastatin depresses blood clotting by inhibiting activation of prothrombin, factor V, and factor XIII and by enhancing factor Va inactivation. Circulation. 2001;103:2248-2253.
62. Zolcinski M, Ciesla-Dul M, Undas A. Effects of atorvastatin on plasma fibrin clot properties in apparently healthy individuals and patients with previous venous thromboembolism. Thromb Haemost. 2012;107:1180-2.

63. Dangas G, Badimon JJ, Smith DA, et al. Pravastatin therapy in hyperlipidemia: effects on thrombus formation and the systemic hemostatic profile. J Am Coll Cardiol. 1999;33:1294-304.
64. Sahebkar A, Serban C, Ursoniu S, et al. The impact of statin therapy on plasma levels of von Willebrand factor antigen. Systematic review and meta-analysis of randomised placebo-controlled trials. Thromb Haemost. 2016;115:520-32.
65. Sahebkar A, Serban C, Mikhailidis DP, et al. Association between statin use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials. Thromb Haemost. 2015;114:546-57.
66. Barreto AC, Maeda NY, Soares RP, et al. Rosuvastatin and vascular dysfunction markers in pulmonary arterial hypertension: a placebo-controlled study. Braz J Med Biol Res. 2008;41:657-63.
67. Adams NB, Lutsey PL, Folsom AR, et al. Statin therapy and levels of hemostatic factors in a healthy population: the Multi-Ethnic Study of Atherosclerosis. J Thromb Haemost. 2013;11:1078-84.
68. Lund JL, Richardson DB, Stürmer T. The active comparator, new user study design in pharmacoepidemiology: historical foundations and contemporary application. Curr Epidemiol Rep. 2015;2:221-228.
69. Kraaijenhagen RA, in 't Anker PS, Koopman MM, et al. High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. Thromb Haemost. 2000;83:5-9.
70. Kyrle PA, Minar E, Hirschl M, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med. 2000;343:457-62.
71. Timp JF, Lijfering WM, Flinterman LE, et al. Predictive value of factor VIII levels for recurrent venous thrombosis: results from the MEGA follow-up study. J Thromb Haemost. 2015;13:1823-32.
72. Cushman M. A new indication for statins to prevent venous thromboembolism? Not yet. J Thromb Haemost. 2009;7:511-3.
73. Eichinger S, Heinze G, Jandeck LM, et al. Risk assessment of recurrence in patients with unprovoked deep vein thrombosis or pulmonary embolism: the Vienna prediction model. Circulation. 2010;121:1630-6.
74. Tosetto A, Iorio A, Marcucci M, et al. Predicting disease recurrence in patients with previous unprovoked venous thromboembolism: a proposed prediction score (DASH). J Thromb Haemost. 2012;10:1019-25.
75. Rodger MA, Scarvelis D, Kahn SR, et al. Long-term risk of venous thrombosis after stopping anticoagulants for a first unprovoked event: A multi-national cohort. Thromb Res. 2016;143:152-8.

CHAPTER 10

Statin use decreases coagulation in users Of vitamin K antagonists

European Journal of Clincal Pharmacology 2016;72:1441-7

Nienke van Rein[1,2], Joseph S. Biedermann[3,4], Stacey M. Bonafacio[1], Marieke J. H. A. Kruip[3,4], Felix J. M. van der Meer[1], Willem M Lijfering[1,2,5]

[1] Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, the Netherlands
[2] Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands
[3] Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands
[4] Star-Medical Diagnostic Center, Rotterdam, The Netherlands
[5] Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

ABSTRACT

Purpose

The purpose of the study is to determine the immediate and long-term effect of statins on coagulation in patients treated with vitamin K antagonists (VKAs).

Methods

We selected patients on VKAs of two Dutch anticoagulation clinics who initiated treatment with a statin between 2009 and 2013. Patients who initiated or stopped concomitant drugs that interact with VKAs or were hospitalized during follow-up were excluded. The VKA dosage (mg/day) after statin initiation was compared with the last VKA dosage before the statin was started. Immediate and long-term differences in VKA dosage (at 6 and 12 weeks) were calculated with a paired student *t* test.

Results

Four hundred thirty-five phenprocoumon users (mean age 70 years, 60% men) and 303 acenocoumarol users (mean age 69 years, 58% men) were included. After start of statin use, the immediate phenprocoumon dosage was 0.02 mg/day (95% CI, 0.00 to 0.03) lower. At 6 and 12 weeks, these phenprocoumon dosages were 0.03 (95% CI, 0.01 to 0.05) and 0.07 mg/day (95% CI, 0.04 to 0.09) lower as compared with the dosage before first statin use. In acenocoumarol users, VKA dosage was 0.04 mg/day (95%CI, 0.01 to 0.07) (immediate effect), 0.10 (95% CI, 0.03 to 0.16) (at 6 weeks) and 0.11 mg/day (95% CI, 0.04 to 0.18) (after 12 weeks) lower.

Conclusions

Initiation of statin treatment was associated with an immediate and long-term minor although statistically significant decrease in VKA dosage in both phenprocoumon and acenocoumarol users, which suggests that statins may have anticoagulant properties.

INTRODUCTION

Patients on vitamin K antagonists (VKAs) often have or develop arterial cardiovascular morbidity, for which they require cardiovascular drugs like statins (1,2). Statins are competitive inhibitors of HMG-CoA reductase that reduce cholesterol biosynthesis, but may also reduce the risk of venous thrombosis (3-5). However, the anticoagulant properties of statins are not well defined and it is unclear how statins could lower the risk of venous thrombosis (5). Currently, three randomised studies and one observational study have been conducted as to observe if statins have anticoagulant properties in VKA users (2, 6–8). These studies showed conflicting results, possibly due to the low number of participants enrolled or because (for the observational study) of residual confounding (2, 6–8). In addition, it is unclear whether potential anticoagulant properties of statins in VKA users are due to drug-drug interactions with VKA or due to pharmacodynamic effects of the statins. To gain more insight into the effects of statins on coagulation in VKA users, we compared coagulation of patients on VKAs before and up till 12 weeks after starting statin therapy.

METHODS

Study design, patient selection, and data collection

Patients' characteristics and outcomes were collected from the computerised records of the anticoagulation clinic Leiden and the Star-Medical Diagnostic Center, Rotterdam. At these anticoagulation clinics, all patients are monitored at least every 6 weeks. At each visit, a standard questionnaire is performed regarding initiation of concomitant medications and planned procedures after which blood is drawn to determine the INR. Based on the INR, the VKA dosage until the next visit is set by a trained physician at the anticoagulation clinic.

All patients, who started treatment with VKAs (i.e. phenprocoumon or acenocoumarol) between January 2009 and December 2013, were screened. This time period was chosen as the coded registration of statin treatment in our databases started in 2009. Patients were included who started to use a statin within this period. Patients were excluded if they started to use inegy (combination of ezetimibe and simvastatin) because it is unknown to which degree both medications are attributable to a possible anticoagulant effect. Patients who were already using statins at baseline or started to use a statin within the first 2 months of VKA treatment were also excluded. Patients were excluded when they were hospitalised between the international normalized ratio (INR) measurement before and after statin initiation because for example an

acute myocardial infarction may affect coagulation and therefore change the outcome (INR and dosage of VKA). Patients were also excluded if they started or stopped any interacting medication with VKAs, according to the national interaction list of Dutch Anticoagulation Clinics, during their individual observation period within this study (9). Neither informed consent nor approval by a medical ethics committee is, according to Dutch law, required for studies in which data are collected from the records by the treating physician.

Outcome measures

INR and dosage of VKAs were determined on the last visit before and the first visit after start of statin use to assess an immediate anticoagulant effect. We determined the immediate difference in INR as this reflects the amount of coagulation at a particular time point. The immediate change in dosage of phenprocoumon or acenocoumarol after first statin use was expected to decrease if statins would increase the INR as VKA dosage is based on INR results. To study the long-term effect of statins on coagulation, the VKA dosage 6 and 12 weeks after statin initiation was compared with the last dosage before statin initiation. If no INR was available at these exact dates, the INR closest to the specific date of statin initiation was chosen. The differences in INR and dosage were also expressed in percentages.

Statistical analysis

Data for continuous variables are expressed as means with standard deviations (SDs), and categorical data are expressed as numbers with percentages. In this study, patients are compared with themselves (cross-over analysis). Therefore, the mean difference in INR and VKA dosage with 95% CI was estimated by means of linear regression and was adjusted for study centre. The reference category in all analyses was the INR and VKA dosage at the last known date before first statin use. All statistical analyses were performed with R version 3.1.1.

RESULTS

Clinical characteristics

Thirty-two thousand, two hundred ninety patients used VKAs between 2009 and 2013, of which 12,074 used phenprocoumon and 20,216 used acenocoumarol. Of these VKA users, 1,273 and 792 initiated a statin during VKA treatment respectively. Statin initiators who were not admitted to a hospital and did not initiate or stop drugs that interact with VKAs during the study period were included for the analysis, resulting in 435 and 303 statin initiators on phenprocoumon and acenocoumarol, respectively.
The mean age of the patients was 70 years (+/- standard deviation 10) when starting statin therapy (Table 1). The most common indication for VKAs was atrial fibrillation (n=537, 73%) and 438 patients (59%) were male. Simvastatin was the most initiated statin (n=516, 70%), while rosuvastatin was not initiated among phenprocoumon users in this sample. One patient started fluvastatin therapy among the phenprocoumon as

Table 1. Clinical characteristics

	Phenprocoumon		Acenocoumarol	
Patients	435		303	
Age	70	(10)	69	(11)
Men	262	(60)	176	(58)
Indication VKA treatment*				
Atrial fibrillation	337	(78)	200	(66)
Venous thrombosis	53	(12)	34	(11)
Mechanical heart valves	13	(3)	24	(8)
Vascular surgery	13	(3)	10	(3)
Ischemic heart disease	20	(5)	23	(8)
Other	12	(3)	1	(0)
Target range INR				
2.5-3.5	404	(93)	242	(80)
3.0-4.0	31	(7)	61	(20)
Type of statin used				
Simvastatin	310	(71)	206	(68)
Atorvastatin	60	(14)	51	(17)
Pravastatin	64	(15)	17	(6)
Rosuvastatin	0	(0)	28	(9)
Fluvastatin	1	(0)	1	(0)

Continuous variables denoted as mean (standard deviation), categorical variables as number (%). * Numbers do not add up to 100% as patients may have multiple indications for VKA treatment.

well as among acenocoumarol users. Clinical characteristics were similar in acenocoumarol and phenprocoumon users and all patients kept the same INR target range during the study period.

Immediate INR and dosage change

Table 2 shows the INRs and mean VKA dose immediately after starting statin treatment in phenprocoumon and acenocoumarol users, respectively. After starting statin treatment, patients had an appointment at the anticoagulation clinic after on average 1 week. The immediate average INR increase in phenprocoumon users was 0.10 (95% CI 0.04 to 0.17), or 6% (95% CI 3 to 8%). In acenocoumarol users no immediate change in INR was observed (INR 0.02 [95% CI -0.10 to 0.14] increased). The mean difference of daily dosage of phenprocoumon users was 0.02 mg per day (95% CI 0.00 to 0.03) lower and for acenocoumarol users 0.04 mg per day (95% CI 0.01 to 0.07) lower. Stratification by statin type showed that both INR changes and dose changes were similar between the different types of statins.

Long-term dosage change

Table 3 shows the long-term change in VKA dosage after initiating statin therapy in acenocoumarol and phenprocoumon users respectively. The mean difference in daily dosage of phenprocoumon users was 0.03 (95% CI 0.01 to 0.05) mg per day lower after 6 weeks and 0.07 (95% CI 0.04 to 0.09) mg per day lower after 12 weeks. The mean difference in daily dosage of acenocoumarol users was 0.10 (95% CI 0.03 to 0.16) mg per day lower after 6 weeks and 0.11 (95% CI 0.04 to 0.18) mg per day lower after 12 weeks. After analyses were stratified by statin type, it appeared that a stronger decrease of VKA dosage was present in simvastatin (among acenocoumarol and phenprocoumon users) and rosuvastatin users (among acenocoumarol users) as compared with the other types of statins.

DISCUSSION

The current study investigated the effect on anticoagulant properties within 738 patients on VKA therapy who initiated statins. Results on immediate INR differences showed that the INR increased slightly in phenprocoumon users but not in acenocoumarol users. The effect of initiating statin treatment on the INR was also investigated in two randomised studies in healthy volunteers (2,6). The study from Yu Cu *et al* showed an INR increase of 0.16 nine days after rosuvastatin initiation, and the study by Jindal *et al* found no INR difference seven days after rosuvastatin initiation

(2,6). The results of our study confirm the results of these trials where the immediate INR increase was also close to null. In addition, the VKA dosage decreased in both phenprocoumon and acenocoumarol users which became apparent for both VKAs after 6 to 12 weeks. The results showed that initiating statin treatment is associated with a decrease of VKA dosage after 6 and 12 weeks, which suggests that statins interact with VKAs or have anticoagulant properties.

A potential explanation for the decrease of VKA dosage in statin users is confounding. However, we did take confounding into account, as we compared patients with themselves in which, confounding by fixed (constant) characteristics (e.g. diabetes mellitus, hypertension and genetics) is eliminated. At the time that this study was conducted, INR ranges in the Netherlands were higher as compared to international guidelines. Because patients are compared with themselves and the INR target range stayed the same during the study period, INR target range could not have confounded our results. However, transient risk factors can introduce (non-fixed) confounding (10). For example, one non-fixed confounding factor is the initiation of concomitant medication or experiencing a cardiovascular event (e.g. a myocardial infarction). To avoid this type of non-fixed confounding, we excluded all patients who started or stopped medications that interact with VKAs during the study period or were admitted to the hospital. Another non-fixed confounding factor is an acute transient disease for example fever (11). However, such a transient disease is unlikely to explain the long-term (6-12 weeks) effect that statins had on the VKA dosage in our study. A further possibility for the decrease in VKA dosage that we found after statin was initiated is that statins interact with VKAs. Acenocoumarol and phenprocoumon are racemic mixtures where the enantiomer largely responsible for the anticoagulant effect are metabolised by CYP3A4 and CYP2C9 (12). Stratification by type of statin showed that rosuvastatin and simvastatin were associated with the strongest decrease in VKA dosage. Rosuvastatin is only 10% metabolised by CYP2C9, while simvastatin is metabolised by CYP3A4 (13). The dosage decrease after initiation of rosuvastatin, which is hardly metabolised by CYP2C9, suggests that our results are not likely to be explained by drug-drug interactions. In addition, differences in lipophilicity of statins are also unlikely to account for the differences found between statins as rosuvastatin is hydrophilic while simvastatin is lipophilic. A potential other explanation is that statins do reduce coagulation, which was suggested by Sahebkar et al. because D-dimer levels decreased after three months of statin therapy and because D-dimer levels are markers of coagulation (14). To get more insight whether simvastatin and rosuvastatin have anticoagulant properties, a next step would be to investigate the effect of these statins on coagulation in patients not on VKAs.

Table 2. Immediate effect on INR and dosage after initiation of statin in VKA users

		Mean INR	(95% CI)	Mean diff. INR	(95% CI)	Percentage difference	(95% CI)
Phenprocoumon							
Any statin							
Last date before start statin use	n=435	2.96	(2.72 to 3.20)	Reference		Reference	
First date after start statin use	n=435	3.15	(2.86 to 3.43)	0.10	(0.04 to 0.17)	6	(3 to 8)
Simvastatin							
Last date before start statin use	n=310	3.03	(2.76 to 3.31)	Reference		Reference	
First date after start statin use	n=310	3.18	(2.84 to 3.53)	0.13	(0.05 to 0.22)	6	(4 to 9)
Atorvastatin							
Last date before start statin use	n=60	2.63	(1.85 to 3.41)	Reference		Reference	
First date after start statin use	n=60	2.72	(2.02 to 3.42)	-0.01	(-0.17 to 0.16)	3	(-4 to 9)
Pravastatin							
Last date before start statin use	n=64	2.83	(2.69 to 2.98)	Reference		Reference	
First date after start statin use	n=64	2.89	(2.73 to 3.05)	0.06	(-0.10 to 0.21)	4	(-2 to 9)
Acenocoumarol							
Any statin							
Last date before start statin use	n=303	2.91	(2.80 to 3.02)	Reference		Reference	
First date after start statin use	n=303	3.04	(2.88 to 3.20)	0.02	(-0.10 to 0.14)	4	(0 to 9)
Simvastatin							
Last date before start statin use	n=206	2.92	(2.78 to 3.05)	Reference		Reference	
First date after start statin use	n=206	3.06	(2.87 to 3.24)	0.02	(-0.11 to 0.17)	4	(0 to 9)
Atorvastatin							
Last date before start statin use	n=51	2.92	(2.62 to 3.21)	Reference		Reference	
First date after start statin use	n=51	2.94	(2.51 to 3.37)	-0.01	(-0.34 to 0.32)	6	(-7 to 19)
Pravastatin							
Last date before start statin use	n=17	2.89	(2.54 to 3.24)	Reference		Reference	
First date after start statin use	n=17	3.09	(2.46 to 3.73)	0.17	(-0.37 to 0.70)	8	(-12 to 28)
Rosuvastatin							
Last date before start statin use	n=28	3.15	(2.90 to 3.40)	Reference		Reference	
First date after start statin use	n=28	3.15	(2.88 to 3.43)	0.01	(-0.30 to 0.31)	3	(-7 to 13)

	Mean dosage mg/day	(95% CI)	Mean diff. (mg/day)	(95% CI)	Percentage difference	(95% CI)
n=435	1.91	(1.58 to 2.24)	Reference		Reference	
n=435	1.88	(1.55 to 2.21)	-0.02	(-0.03 to 0.00)	-1	(-1 to 0)
n=310	2.10	(1.70 to 2.49)	Reference		Reference	
n=310	2.06	(1.68 to 2.45)	-0.02	(-0.03 to -0.01)	-1	(-1 to -1)
n=60	1.29	(0.33 to 2.26)	Reference		Reference	
n=60	1.29	(0.35 to 2.23)	-0.01	(-0.03 to 0.01)	0	(-1 to 1)
n=64	2.10	(1.90 to 2.30)	Reference		Reference	
n=64	2.10	(1.89 to 2.30)	0.00	(-0.02 to 0.01)	0	(-1 to 0)
n=303	2.66	(2.45 to 2.86)	Reference		Reference	
n=303	2.63	(2.42 to 2.83)	-0.04	(-0.07 to -0.01)	-1	(-3 to 0)
n=203	2.69	(2.46 to 2.93)	Reference		Reference	
n=203	2.66	(2.42 to 2.90)	-0.04	(-0.08 to -0.01)	-2	(-3 to 0)
n=51	2.71	(2.12 to 3.30)	Reference		Reference	
n=51	2.68	(0.35 to 2.23)	-0.02	(-0.06 to 0.03)	-2	(-3 to 0)
n=17	2.04	(1.54 to 2.53)	Reference		Reference	
n=17	2.00	(1.54 to 2.45)	-0.06	(-0.33 to 0.21)	3	(-19 to 26)
n=28	3.04	(2.49 to 3.60)	Reference		Reference	
n=28	3.02	(2.47 to 2.57)	-0.02	(-0.04 to 0.00)	-1	(-1 to 0)

Table 3. Long term effect of statin initiation on VKA dosage

		Mean dosage mg/day	(95% CI)	Mean difference dosage (mg/day)	(95% CI)	Mean difference dosage percentage	(95% CI)
Phenprocoumon							
Any statin							
Last date before start statin use	n=435	1.91	(1.58 to 2.24)	Reference		Reference	
6 weeks after start statin use	n=434	1.85	(1.52 to 2.17)	-0.03	(-0.05 to -0.01)	-1	(-2 to 0)
12 weeks after start statin use	n=408	1.81	(1.49 to 2.13)	-0.07	(-0.09 to -0.04)	-3	(-4 to -1)
Simvastatin							
Last date before start statin use	n=310	2.10	(1.70 to 2.49)	Reference		Reference	
6 weeks after start statin use	n=309	2.02	(1.63 to 2.40)	-0.04	(-0.07 to -0.02)	-2	(-3 to 0)
12 weeks after start statin use	n=388	1.97	(1.60 to 2.34)	-0.08	(-0.11 to -0.04)	-3	(-4 to -1)
Atorvastatin							
Last date before start statin use	n=60	1.29	(0.33 to 2.26)	Reference		Reference	
6 weeks after start statin use	n=60	1.29	(0.33 to 2.25)	0.00	(-0.03 to 0.03)	0	(-1 to 1)
12 weeks after start statin use	n=57	1.28	(0.31 to 2.26)	-0.02	(-0.07 to 0.02)	-1	(-3 to 0)
Pravastatin							
Last date before start statin use	n=64	2.10	(1.90 to 2.30)	Reference		Reference	
6 weeks after start statin use	n=64	2.02	(1.88 to 2.30)	-0.01	(-0.04 to 0.01)	-1	(-2 to 0)
12 weeks after start statin use	n=62	2.06	(1.85 to 2.27)	-0.05	(-0.09 to -0.02)	-3	(-5 to -1)

Table 3. *Continued*

		Mean dosage mg/day	(95% CI)	Mean difference dosage (mg/day)	(95% CI)	Mean difference dosage percentage	(95% CI)
Acenocoumarol							
Any statin							
Last date before start statin use	n=303	2.66	(2.45 to 2.86)	Reference		Reference	
6 weeks after start statin use	n=303	2.64	(2.44 to 2.84)	-0.10	(-0.16 to -0.03)	-2	(-4 to 1)
12 weeks after start statin use	n=300	2.63	(2.42 to 2.84)	-0.11	(-0.18 to -0.04)	-2	(-5 to 1)
Simvastatin							
Last date before start statin use	n=206	2.70	(2.46 to 2.93)	Reference		Reference	
6 weeks after start statin use	n=206	2.62	(2.39 to 2.86)	-0.14	(-0.22 to -0.06)	-4	(-6 to 0)
12 weeks after start statin use	n=204	2.59	(2.35 to 2.84)	-0.17	(-0.26 to -0.07)	-4	(-7 to -1)
Atorvastatin							
Last date before start statin use	n=51	2.71	(2.12 to 3.30)	Reference		Reference	
6 weeks after start statin use	n=51	2.99	(2.44 to 3.54)	0.02	(-0.11 to 0.16)	2	(-5 to 9)
12 weeks after start statin use	n=50	3.20	(2.53 to 3.86)	0.08	(-0.08 to 0.24)	4	(-5 to 12)
Pravastatin							
Last date before start statin use	n=17	2.04	(1.54 to 2.53)	Reference		Reference	
6 weeks after start statin use	n=17	2.05	(1.60 to 2.51)	0.02	(-0.26 to 0.31)	9	(-16 to 35)
12 weeks after start statin use	n=17	2.04	(1.59 to 2.51)	0.02	(-0.30 to 0.33)	10	(-18 to 39)
Rosuvastatin							
Last date before start statin use	n=28	3.04	(2.49 to 3.60)	Reference		Reference	
6 weeks after start statin use	n=28	2.97	(2.45 to 3.50)	-0.07	(-0.12 to -0.02)	-1	(-3 to 0)
12 weeks after start statin use	n=28	2.94	(2.43 to 3.44)	-0.10	(-0.20 to -0.01)	-2	(-6 to 0)

Though the current study is of etiological interest as it gives a lead why statins might be able to decrease venous thrombosis risk, its clinical effect appears to be minimal: INRs did not increase immediately and only marginally, and the VKA dose reduction was also minimal.

A potential limitation of our study is that co-medication was self-reported and the only statin reported by the pharmacy to the anticoagulation clinics was rosuvastatin. Consequently there may be discrepancies between the medication records of the anticoagulation clinics and what the patients used. As patients were compared with themselves, we expect that this has not influenced the results. An additional limitation is that we excluded patients who were hospitalised between the INR measurement before and after statin initiation. We did this because the assumption of the study, that there are no other environmental changes present that can affect VKA dosage and/or INR in the patient except that the patient started with statin, is otherwise not held. For that reason we could have missed patients of more 'dramatic' changes of anticoagulation, like patients with a major bleed. Furthermore, pharmacokinetics of the two studied VKAs do differ, for example phenprocoumon has a longer half-life as compared with acenocoumarol (12). However, differences in pharmacokinetics of the VKAs tested are unlikely to have contributed to the statin results found in this study as results were similar in both acenocoumarol- and phenprocoumon users. Another limitation is that we assumed that patients are compliant to their statin therapy. It is likely that not all patients were fully compliant as previous studies showed an average adherence to statins of 71-77% (15). Our results could therefore be diluted and the effects on VKA dosage are likely to be stronger if we could have taken statin adherence into close account. A final limitation of our study is that the dosage of statins was not registered in the electronic system. Therefore, no analyses could be performed that took the dosage of statin into account.

In conclusion, we found that statin treatment was associated with a minor although statistically significant decrease in VKA dosage in both phenprocoumon and acenocoumarol users, which suggests that statins may have anticoagulant properties.

REFERENCES

1. Nelson WW, Choi JC, Vanderpoel J, Damaraju CV, Wildgoose P, Fields LE, et al. Impact of co-morbidities and patient characteristics on international normalized ratio control over time in patients with nonvalvular atrial fibrillation. Am J Cardiol 2013;112(4):509-12.
2. Yu CY, Campbell SE, Zhu B, Knadler MP, Small DS, Sponseller CA, et al. Effect of pitavastatin vs. rosuvastatin on international normalized ratio in healthy volunteers on steady-state warfarin. Curr Med Res Opin 2012;28(2):187-94.
3. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science 2001;292(5519):1160-4.
4. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med 2009;360(18):1851-61.
5. Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC. Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. Semin Thromb Hemost 2001;37(8):885-96.
6. Jindal D, Tandon M, Sharma S, Pillai K. Pharmacodynamic evaluation of warfarin and rosuvastatin co-administration in healthy subjects. Eur J Clin Pharmacol 2005;61(9):621-5.
7. Simonson SG, Martin PD, Mitchell PD, Lasseter K, Gibson G, Schneck DW. Effect of rosuvastatin on warfarin pharmacodynamics and pharmacokinetics. J Clin Pharmacol 2005;45(8):927-34.
8. van Schie RM, Verhoef TI, Boejharat SB, Schalekamp T, Wessels JA, le Cessie S, et al. Evaluation of the effect of statin use on the acenocoumarol and phenprocoumon maintenance dose. Drug Metabol Drug Interact 2012;27(4):229-34.
9. Http://www.fnt.nl/behandeling/cumarine-interacties.html;visited 22–7-2015.
10. Greenland S. Confounding and exposure trends in case-crossover and case-time-control designs. Epidemiology 1996;7(3):231-9
11. Marie I, Leprince P, Menard JF, Tharasse C, Levesque H. Risk factors of vitamin K antagonist overcoagulation. QJM 2012;105(1):53-62.
12. Beinema M, Brouwers JR, Schalekamp T, Wilffert B. Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon. Thromb Haemost 2008;100(6):1052-7.
13. Gryn SE, Hegele RA. Pharmacogenomics, lipid disorders, and treatment options. Clin Pharmacol Ther 2014;96(1):36-47.
14. Sahebkar A, Serban C, Mikhailidis DP, Undas A, Lip GY, Muntner P, et al. Association between statins use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials. Thromb Haemost 2015;114(3):546-57
15. Gagne JJ, Choudhry NK, Kesselheim AS, Polinski JM, Hutchins D, Matlin OS, et al. Comparative effectiveness of generic and brand-name statins on patient outcomes: a cohort study. Ann Intern Med 2014;161(6):400-7

CHAPTER 11

Platelet reactivity in patient with venous thrombosis who use rosuvastatin: a randomized controlled clinical trial

Journal of Thrombosis and Haemostasis 2016;14:695-703

Joseph S. Biedermann[1], Suzanne C. Cannegieter[2-4], Mark Roest[5,6], Felix J.M. van der Meer[3], Pieter H. Reitsma[4], Marieke J.H.A. Kruip[1], Willem M. Lijfering[2-4]

[1] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[2] Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands
[3] Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, the Netherlands
[4] Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, the Netherlands
[5] Department of Clinical Chemistry and Hematology, University Medical Center Utrecht, the Netherlands
[6] Synapse B.V., Maastricht University, Maastricht, the Netherlands

ABSTRACT

Background

Statins may exert a protective effect to the risk of venous thrombosis (VT), but the mechanism is unclear.

Objectives

In this open label, randomized clinical trial (www.clinicaltrials.gov NCT01613794), we aimed to determine the *ex vivo* effect of rosuvastatin on platelet reactivity in patients with a history of VT.

Methods

Platelet reactivity, in platelet reaction units (PRU), was measured at baseline and after 28 days with the VerifyNow, which uses arachidonic acid to determine thromboxane-mediated platelet aggregation, in 50 consecutive patients included in our study (25 receiving rosuvastatin and 25 without intervention).

Results

Forty-seven out of 50 (94.0%) consecutively enrolled patients had 2 valid PRU measurements. Mean PRU in rosuvastatin users was 609 at baseline and 613 at end of study (mean change 5; 95% CI, -18 to 27). Mean PRU in non- users was 620 at baseline and 618 at end of study (mean change -2; 95% CI, -15 to 12). The mean difference in PRU change between users and non-users was 6 (95% CI, -20 to 33). Excluding patients who used antiplatelet medication, or had thrombocytopenia, revealed similar results, i.e. no apparent effect of rosuvastatin on PRU with a mean difference in PRU change between users and non-users of -1 (95% CI, -20 to 19).

Conclusions

Rosuvastatin does not affect platelet reactivity using arachidonic acid as agonist in patients with a history of VT.

INTRODUCTION

Currently, the only effective strategy to prevent recurrent venous thrombosis is to continue anticoagulant therapy indefinitely (1). In view of the substantial risk of bleeding complications during such long-term use, other treatment modalities are urgently needed (2). That statins may exert a protective effect to venous thrombosis risk was first sparked in 2000, but remains controversial (3-4). Results from studies suggest a 20-50% lower risk of venous thrombosis for patients using statins, with a greater risk reduction for rosuvastatin as compared with other statin types (5-8). However, the mechanism remains unclear (9). Dyslipidemia may be the most plausible culprit to be considered. However, as dyslipidemia is not related to an increased risk of venous thrombosis, and because non-statin lipid lowering drugs (i.e. fibrates) do not reduce venous thrombosis risk, it is unlikely that statins decrease venous thrombosis risk by lipid lowering activities (2,10). Another mechanism through which statins may decrease the risk of venous thrombosis is by inhibiting platelet activation and consequently aggregation (11-13). Animal models have shown that platelet activation plays a key role in the initiation of thrombus formation in deep vein thrombosis (11,12). Furthermore, *in vitro* studies indicate that statins inhibit platelet activation via several lipid-independent mechanisms including the inhibition of thromboxane A2 (TxA2) formation (14). Although inhibitory effects of statins on platelet function assessed by *ex vivo* tests of platelet aggregation have been described in patients at risk for cardiovascular disease, the effects of statins on platelet function in patients with a history of venous thrombosis is unclear (15-17). Since enhanced platelet aggregation has been reported in patients with venous thrombosis, and the VerifyNow assay can measure TxA2-mediated platelet activation and aggregation, we decided to investigate the effect of rosuvastatin on platelet aggregation in patients with a history of confirmed deep vein thrombosis or pulmonary embolism as measured by the VerifyNow assay (18).

METHODS

Design

Data from the STAtins Reduce Thrombophilia (START) study were used. START is a randomized, controlled, open label, clinical trial on whether the coagulation profile in persons with a history of objectively confirmed venous thrombosis will improve when using rosuvastatin. START is approved by the Medical Ethics Committee of the Leiden University Medical Center, and registered at www.clinicaltrials.gov as NCT01613794.

Participants

Patients aged >18 years with confirmed symptomatic deep vein thrombosis or pulmonary embolism who completed 3 to 12 months of treatment with vitamin K antagonists, and who were allowed to stop anticoagulant treatment by their treating physician were eligible for this trial. The study baseline visit was at the time of the last regular visit of the patient to the anticoagulation clinic. After written informed consent, participants were screened on acquired risk factors for thrombosis through a questionnaire and tested on blood parameters that may exclude a patient from taking rosuvastatin. After a time window of 28 days (+/- 5 days) (to allow a wear off of anticoagulant drugs), a blood sample was drawn at randomization visit, and at time of stopping rosuvastatin 28 days later. At randomization, participants were allocated to receive either rosuvastatin 20 mg/day for 28 days or no study medication. Blood samples were drawn at randomization visit and at the end of the 28-day study period. All blood drawings were performed between 08:30 am and 14:00 pm and at the same time of day. Platelet reactivity was measured using the VerifyNow Assay (Accumetrics, San Diego, CA, USA). The VerifyNow assay is a point-of-care device that measures thromboxane A2-mediated platelet activation, in platelet rich plasma, using an optical turbidimetry method and converts this into Platelet Reaction Units (PRU). In accordance with the manufacturer's instructions, platelet reactivity was measured in citrated whole blood within 4 hours after venipuncture.

Statistical analysis

The general characteristics of rosuvastatin users and non-users are reported as means and ranges. Mean PRU with 95% confidence interval (95% CI) were calculated at time of randomization and at the end of the study period. All analyses were performed by intention to treat. Changes in platelet reactivity were expressed as the mean difference (with 95% CI) in PRU at the end of the study period in rosuvastatin users versus non-users. Since platelet reactivity was measured at two different time points (i.e. at randomization visit and at the end of study visit), we evaluated the change in platelet reactivity at an individual level. Mean within person changes in PRU were analyzed using a paired samples t-test in both rosuvastatin and non-rosuvastatin users. Mean difference in PRU change between rosuvastatin and non-rosuvastatin users was assessed using an independent t-test. Linear regression analysis was used to adjust mean PRU differences between rosuvastatin and non-rosuvastatin users for age, sex and type of index event (provoked or unprovoked). Since use of concomitant medication was known for each patient, a restriction analysis was performed excluding participants using antiplatelet drugs, or participants with known thrombocytopenia. Based on a standard deviation (SD) of 20 PRU, the study was powered to find a difference in PRU

change of 12 between 25 statin users vs. 25 non-users, with a 2 sided alpha=0,05 and 80% power (19). We considered this as sufficiently powered to exclude a possible type II error, since a decrease in platelet aggregation in individuals who start to use aspirin is on average 200 PRU (19). All analyses were performed using SPSS version 21.0 (IBM Corp. Armonk, NY, USA).

RESULTS

The participants were included between September 2013-September 2014. For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed on PRU, is provided in Figure 1. We took 100 blood samples from 50 enrolled participants. During the 2 time points, 3 samples (3%) were unavailable for analysis: 2 were missing because of technical failure and 1 participant

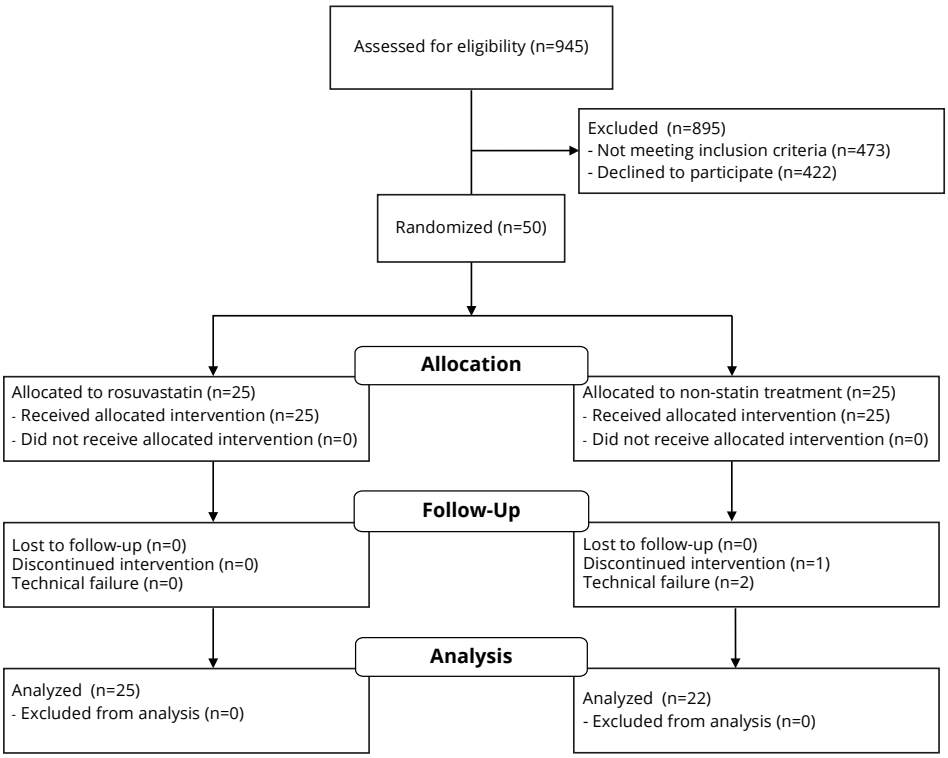


Figure 1. Flow diagram of eligible and analyzed START participants

was unable to deliver a second blood test at end of follow-up as he encountered a recurrent venous thrombotic event. During follow-up 47 out of 50 (94.0%) participants had valid PRU measurements at baseline and at the end of study (25 rosuvastatin users and 22 non-users). Their clinical characteristics are shown in Table 1. Mean age at enrollment was 59 years, and the majority were men. Two of the rosuvastatin users used antiplatelet drugs during the study period and 1 of the non-users had thrombocytopenia. The mean total cholesterol level in participants allocated to rosuvastatin treatment was 5.6 (3.4-9.0) mmol/L (range) at baseline and 3.6 (2.3-5.5) mmol/L (range) at end of follow-up (mean difference -2.0, 95% CI, -2.3 to -1.7). For the non-users these mean levels were 5.4 (3.5-7.7) mmol/L (range) at baseline and 5.1 (3.6 to 6.5) mmol/L (range) at end of follow-up.

The effect of rosuvastatin therapy on platelet reactivity is shown in Table 2. Mean PRU in rosuvastatin users was 609 at baseline and 613 at end of follow-up (mean PRU change 5; 95% CI, -18 to 27). Mean PRU in non-users was 620 at baseline and 618 at end of follow-up (mean PRU change -2; 95% CI, -15 to 12). The mean difference in PRU change between rosuvastatin users and non-users was 7 (95% CI, -20 to 33). Restriction analysis showed similar results, i.e. no difference in PRU change between rosuvastatin users and non-users (mean difference in PRU change -1; 95% CI, -20 to 19). Adjustment for age, sex and type of index event (provoked/unprovoked) did not change these results (mean difference in PRU change 1; 95% CI, -19 to 20). PRU change per participant in rosuvastatin users and non-users is shown in Figure 2. As shown in this figure, most participants had a PRU change that was close to 0. Also the number of rosuvastatin users in whom the end of study PRU measurement was lower than the baseline PRU (n=14, 56%) was similar as in the non-users (n=11, 50%).

Table 1. Clinical characteristics

	Rosuvastatin users (n=25)	**Non-users (n=22)**
Mean age (range), y	59 (33-77)	59 (21-80)
Male, n (%)	17 (68)	18 (82)
Type of venous thrombosis, n (%)		
Deep vein thrombosis	17 (68)	10 (45)
Pulmonary embolism	8 (32)	12 (55)
Unprovoked, n (%)	13 (52)	9 (41)
Provoked, n (%)	12 (48)	13 (59)
Concomitant antiplatelet drug use	2 (8)	0 (0)
Known thrombocytopenia, n (%)	0 (0)	1 (5)

Table 2. Effect of rosuvastatin therapy on platelet reactivity

	Mean PRU (range)				
	Baseline	End of study	Mean change (95% CI)	Mean difference in change (95% CI)	Adjusted mean difference in change (95% CI) *
Overall					
Non-users (n=22)	620	618	-2 (-15 to 11)	Reference	Reference
Rosuvastin users (n=25)	609	613	5 (-18 to 28)	7 (-20 to 33)	14 (-13 to 40)
Restriction analysis †					
Non-users (n=21)	628	624	-4 (-17 to 10)	Reference	Reference
Rosuvastin users (n=23)	624	619	-5 (-19 to 10)	-1 (-20 to 19)	1 (-19 to 20)

* Adjusted for age, sex and index VTE event (provoked/unprovoked). † Excluding participants who use antiplatelet drugs at or during follow-up (n=2), had known thrombocytopenia (n=1), or had extreme outliers (n=1). CI denotes confidence interval; PRU: platetelet reaction units.

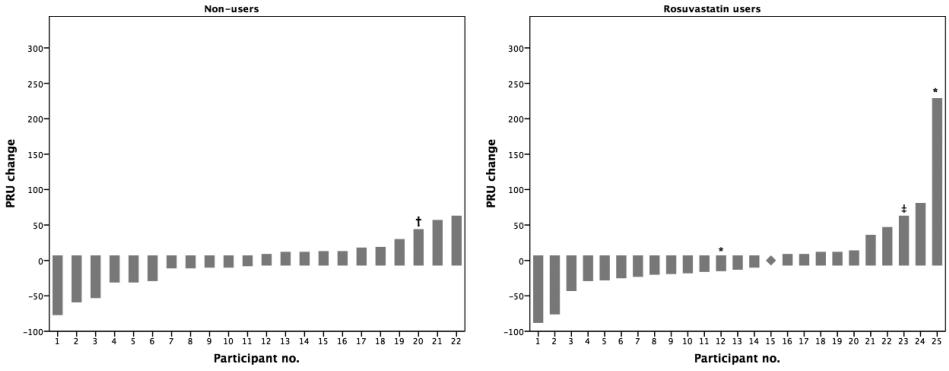


Figure 2. Change in platelet reaction units (PRU) in rosuvastatin users and non-users
* Participant on antiplatelet drugs at or during follow-up (n=2)
† Participant with thrombocytopenia (n=1)

DISCUSSION

We observed that rosuvastatin 20mg daily for 28 days did not affect arachidonic acid-mediated platelet aggregation as measured by the VerifyNow assay. Therefore, our findings show that it is unlikely that the association of a decreased risk of venous thrombosis in rosuvastatin users is explained by decreased arachidonic acid-mediated thromboxane synthesis. However, a limitation of our study is that only the VerifyNow assay was used to evaluate platelet function. Therefore we cannot exclude potential other antiplatelet effects as can be measured using different platelet function tests

(20). Another limitation of our study is that, due to the relatively small sample size, subgroup analyses were not performed. However, within-person changes in platelet reactivity were compared between exposures, which at least eliminates fixed-confounding factors by design. Furthermore, adjustments for age, sex and type of index event, yielded similar results, i.e. no difference in platelet reactivity change between rosuvastatin users and non-users. There is a possibility, however, that platelets behave differently in patients with a high thrombotic potential, which unfortunately we could not further investigate due to small numbers (21). Similarly, we cannot rule out the possibility that patients who were not willing to participate in this trial had a higher thrombotic potential, yielding different results regarding platelet reactivity in rosuvastatin users than we observed.

Apart from an antiplatelet effect, several other pleiotropic effects of statins on coagulation have been described, including inhibition of tissue factor synthesis, increased thrombomodulin formation and enhanced fibrinolysis (22). The magnitude of contribution of these pleiotropic effects of statins on venous thrombosis risk is poorly understood and is beyond the scope of the current study. This needs further exploration for which we are currently continuing to enrol patients in our START trial (registered at NCT01613794).

In conclusion, in this randomized controlled trial, rosuvastatin used for 28 days did not affect platelet reactivity using arachidonic acid as agonist in patients with a history of confirmed deep vein thrombosis or pulmonary embolism.

REFERENCES

1. Kearon C, Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ, et al. Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e419S-94S.
2. Kyrle PA, Rosendaal FR, Eichinger S. Risk assessment for recurrent venous thrombosis. Lancet. 2010;376(9757):2032-9.
3. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, et al. Postmenopausal hormone therapy increases the risk for venous thromboembolic disease. The Heart and Estrogen/progestin Replacement Study. Ann Intern Med. 2000;132(9):689-96.
4. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? PLoS Med. 2012;9(9):e1001311
5. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360(18):1851-61
6. Ramcharan AS, Van Stralen KJ, Snoep JD, Mantel-Teeuwisse AK, Rosendaal FR, Doggen CJ. HMG-CoA reductase inhibitors, other lipid-lowering medication, antiplatelet therapy, and the risk of venous thrombosis. J Thromb Haemost. 2009;7(4):514-20
7. Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. BMJ. 2010;340:c2197
8. Rahimi K, Bhala N, Kamphuisen P, Emberson J, Biere-Rafi S, Krane V, et al.Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. PLoS Med. 2012;9(9):e1001310.
9. Cushman M. A new indication for statins to prevent venous thromboembolism? Not yet. J Thromb Haemost. 2009;7(4):511-3.
10. Glynn RJ, Rosner B. Comparison of risk factors for the competing risks of coronary heart disease, stroke, and venous thromboembolism. Am J Epidemiol. 2005;162(10):975-82.
11. Brill A, Fuchs TA, Chauhan AK, Yang JJ, De Meyer SF, Köllnberger M, et al. von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. Blood. 2011;117(4):1400-7.
12. Joglekar MV, Ware J, Xu J, Fitzgerald ME, Gartner TK. Platelets, glycoprotein Ib-IX, and von Willebrand factor are required for FeCl(3)-induced occlusive thrombus formation in the inferior vena cava of mice. Platelets. 2013;24(3):205-12.
13. Puccetti L, Santilli F, Pasqui AL, Lattanzio S, Liani R, Ciani F, et al. Effects of atorvastatin and rosuvastatin on thromboxane-dependent platelet activation and oxidative stress in hypercholesterolemia. Atherosclerosis. 2011;214(1):122–8.
14. Pignatelli P, Carnevale R, Pastori D, Cangemi R, Napoleone L, Bartimoccia S, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. Circulation. 2012;126(1):92–103.
15. Pignatelli P, Sanguigni V, Lenti L Loffredo L, Carnevale R, Sorge R, et al. Oxidative stress-mediated platelet CD40 ligand upregulation in patients with hypercholesterolemia: effect of atorvastatin. J Thromb Haemost.2007;5(6):1170–8.
16. Blann AD, Gurney D, Hughes E, Buggins P, Silverman SH, Lip GY. Influence of pravastatin on lipoproteins, and on endothelial, platelet, and inflammatory markers in subjects with peripheral artery disease. Am J Cardiol. 2001;88(1):A7–A8, 89-92.
17. Alber HF, Frick M, Suessenbacher A, Doerler J, Schirmer M, Stocker EM, et al. Effect of atorvastatin on circulating proinflammatory T-lymphocyte subsets and soluble CD40 ligand in patients with stable coronary artery disease: a randomized, placebo-controlled study. Am Heart J. 2006;151(1):139.
18. Weber M, Gerdsen F, Gutensohn K, Schoder V, Eifrig B, Hossfeld DK. Enhanced platelet aggregation with TRAP-6 and collagen in platelet aggregometry in patients with venous thromboembolism. Thromb Res. 2002;107(6):325-8.

19. Bonten TN, Saris A, van Oostrom MJ, Snoep JD, Rosendaal FR, Zwaginga J, et al. Effect of aspirin intake at bedtime versus on awakening on circadian rhythm of platelet reactivity. A randomised cross-over trial. Thromb Haemost. 2014;112(6): 1209-18.
20. Israels SJ. Laboratory testing for platelet function disorders. Int J Lab Hematol. 2015; 37 Suppl 1:18-24
21. Pettersen AA, Arnesen H, Opstad TB, Bratseth V, Seljeflot I. Markers of endothelial and platelet activation are associated with high on-aspirin platelet reactivity in patients with stable coronary artery disease. Thromb Res. 2012;130(3):424-8.
22. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. Circulation 2013;127(2):251-7.

CHAPTER 12

Rosuvastatin use improves measures of coagulation in patients with venous thrombosis

Submitted Manuscript

Joseph. S. Biedermann[1,2], Marieke J.H.A. Kruip[1,2], Felix J. van der Meer[3], Frits R. Rosendaal[4], Frank W.G. Leebeek[1], Suzanne C. Cannegieter[3,4], Willem M. Lijfering[4]

[1] Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands
[2] Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
[3] Department of Thrombosis and Haemostasis, Leiden University Medical Center, the Netherlands
[4] Star-Medical Anticoagulation Clinic, Rotterdam, the Netherlands

ABSTRACT

Background

Observational studies indicate that statins reduce the risk of recurrent venous thrombosis, without increasing the risk of bleeding complications. However, trials have not been performed and the mechanism is unknown. Therefore, the aim of the STAtins Reduce Thrombophilia (START) trial was to investigate whether statin therapy improves the coagulation profile in patients with a history of venous thrombosis.

Methods

Randomized clinical trial (NCT01613794). Patients with prior venous thrombosis, who recently discontinued anticoagulant therapy, were randomized to rosuvastatin 20 mg/day for 4 weeks or no intervention. Blood was drawn at baseline and at end of study. The primary outcome (for which the study was powered) was factor VIII:C. In total 5 coagulation factors were measured: factor (F) VIII:C, von Willebrand factor (VWF:Ag), FVII:C, FXI:C and D-dimer.

Results

Among 247 randomized participants, mean age was 58 years, 62% were women, and 49% had unprovoked venous thrombosis. For all tested coagulation factors, the mean difference in change was lower or tended to be lower at end of study in rosuvastatin users versus non-users. Results were most consistent for FVIII:C. In rosuvastatin users, mean FVIII:C levels were 7.2 IU/dL (95% CI, 2.9 to 11.5)lower, while among non-users, no change in F VIII:C was observed (mean difference -0.1; 95% CI, -3.0 to 2.9). The mean age and sex adjusted difference in FVIII:C change was -6.7 IU/dL (95% CI, -12.0 to -1.4) in rosuvastatin users versus non-users. Subgroup analyses revealed similar results as in the main analysis, with the exception of participants with unprovoked venous thrombosis or in those with cardiovascular risk factors. In these participants the decrease in coagulation factors by rosuvastatin was more pronounced than in provoked venous thrombosis or absence of cardiovascular risk factors, respectively.

Conclusions

Rosuvastatin 20 mg/day improved the coagulation profile among patients with prior venous thrombosis.

INTRODUCTION

Venous thrombosis, the collective term for deep vein thrombosis of the leg, pulmonary embolism or both, is the third most common vascular disease after myocardial infarction and ischemic stroke (1). Venous thrombosis affects 1-2 per thousand people per year, has a 2.6% immediate death rate and recurrence rates of 25% within 5 years (2). Currently, the only effective strategy to prevent recurrent events is to continue anticoagulation indefinitely (3). In view of bleeding complications, safer options to reduce the risk of recurrent venous thrombosis are necessary (4). For this statins may form a suitable candidate as statins do not induce bleeding but may reduce the risk of venous thrombosis (4,5). In this aspect, results from observational studies that showed that statins are associated with a 30-50% lower risk of first venous thrombosis seem promising, but often included prevalent statin users in their statin exposure categories (6-8). Such prevalent users can introduce two types of bias: (1) under-ascertainment of events that occur early after starting treatment (survivor bias) and (2) the inability to control for those who do or do not adhere to statin treatment (adherence bias) (9,10). For this matter, it is interesting to note that in a meta-analysis of statin use and risk of first venous thrombosis, only one of the seven mentioned studies included statin initiators in their study (new-user design) and reported an odds ratio of 1.02 (95% confidence interval [CI], 0.88 to 1.18), which is in contrast to the overall odds ratio of 0.62 (95% CI, 0.45 to 0.86) (7,8). This suggests that the observed association between statin use and a decreased risk of first venous thrombosis may have been biased due to the inclusion of prevalent users. However, other studies in which prevalent user bias was excluded by design have been published. In a cohort study of nearly 2 million individuals from the United Kingdom, in which a new-user design was used, the authors showed that rosuvastatin use was associated with a strongly (approximately 40%) reduced risk of venous thrombosis (11). That result closely resembles those from a randomized clinical trial in which apparently healthy individuals were randomized to rosuvastatin or placebo (JUPITER trial) where a 40% risk reduction when using rosuvastatin compared with placebo was found (12). In the absence of other randomized trials with venous thrombosis as the primary end point, Rahimi and colleagues presented a pooled analysis of 29 randomized statin studies in which venous thrombotic events were reported as serious adverse events (13). Although they failed to confirm a risk reduction of venous thrombosis by statin treatment, the authors found that individuals who were randomized to rosuvastatin still had an approximately 40% reduced risk of venous thrombosis (hazard ratio, 0.65; 95% CI, 0.33 to 1.28) (13). Based on a report that showed that statins can reduce factor VIII, one would suspect that statin use protects to some extent against venous thrombosis by downsizing

coagulation activation (14). This finding should be interpreted with caution as it comes from a non-randomized study. Recently published findings from randomized clinical trials of statin therapy suggest some effect of lowering levels of von Willebrand factor and D-dimer (15,16). However, funnel plot analyses revealed that these positive results might be due to publication bias (15,16). Furthermore, no randomized clinical trials have investigated the impact of statin therapy on the risk of recurrent venous thrombosis. Not knowing a clear pathophysiological mechanism behind the supposed causal association between statin use and a reduced risk of recurrent venous thrombosis, may explain the lack of conducting such a costly and endeavoring trial.
Therefore, the aim of the STAtins Reduce Thrombophilia (START) trial was to investigate whether statin therapy improves the coagulation profile in patients with a history of venous thrombosis. For this purpose, the effect of rosuvastatin use on several coagulation markers was assessed. Rosuvastatin 20 mg/day was chosen as the study drug as rosuvastatin consistently showed the strongest association between its intake and a reduced risk of venous thrombosis (11-13).

METHODS

Trial design

START is a multicenter, randomized, controlled, open label, clinical trial that investigates whether the coagulation profile in persons with a history of venous thrombosis will improve when using rosuvastatin. The open label design was chosen as it was considered unlikely that knowledge as to whether a participant received rosuvastatin or non-statin could affect the outcome (change in coagulation) one month later. We undertook the study in accordance with the Declaration of Helsinki and International Conference on Harmonization guidelines for Good Clinical Practice. All participants gave written informed consent prior to participation. START was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands, and is registered at www.clinicaltrials.gov as NCT01613794.

Participants

Participants were recruited at three anticoagulation clinics in the Netherlands (Leiden, Hoofddorp and Rotterdam), which monitor anticoagulant treatment with vitamin K antagonists of patients with venous thrombosis in well-defined geographic areas. Individuals aged 18 years or older with (initial or recurrent) confirmed symptomatic proximal deep vein thrombosis or pulmonary embolism who were allowed to stop oral anticoagulant treatment by their treating physician, were eligible for the study. Reasons

for exclusion were: individuals already using statins or lipid lowering drugs, or any other contraindications for rosuvastatin 20 mg/day use as provided by the instruction leaflet of the manufacturer (17).

Intervention

Informed consent was obtained at the study baseline visit. The study baseline visit was defined at the time of the last regular visit of the patient to the anticoagulation clinic. After informed consent, participants were screened on acquired risk factors for thrombosis through a questionnaire and tested on liver and kidney function. At randomization, participants were allocated to receive either rosuvastatin 20 mg/day or no study medication. Allocating participants to treatment/no treatment was performed using randomisation.

The duration of the study was 28 days. Such a treatment period was considered long enough to identify an effect of rosuvastatin on the coagulation system as some small, non-randomized, studies showed beneficial effects of statins on the coagulation system as early as after a 3-day statin administration (18).

Measurements

After a time window of 28 days (+/- 5 days) when patients stopped using their vitamin K antagonist (to allow a wear off of anticoagulant drugs), a blood sample was drawn at randomization visit and at the end of the 28-days study period. All blood drawings were performed between 08:00 am and 15:00 pm. Blood was collected in tubes containing sodium citrate (3.2%) and centrifuged within 3 hours of venepuncture at 2500 g for 15 minutes at 18°C, after which plasma was immediately stored at -80°C. Laboratory technicians, who were unaware of which participants were rosuvastatin-users, performed the assays after all participants had completed the study.

From these blood samples, levels of coagulation markers that are related to liver function (factor [F] VII:C, FXI:C), and endothelial function (FVIII:C and von Willebrand factor [vWF]:Ag), and one global assay (D-dimer) were assessed (19,20). We decided to use this set of coagulation assays as these could globally indicate if and by which mechanism rosuvastatin reduces thrombophilia.

All laboratory measurements (FVII:C, FVIII:C, FXI:C, D-dimer, VWF:Ag), were analyzed on the ACL-Top 700 analyzer (Instrumentation Laboratory). FVIII:C, and FXI:C levels were measured using modified activated partial thromboplastins time assays using immunodepleted plasmas. Similarly, FVII:C was determined using a modified prothrombin time. VWF:Ag and D-dimer levels were measured using an automated latex enhanced immunoassay using the HemosIL VWF:Ag and the HemosIL D-dimer HS 500 reagent kit, respectively.

Study size

Because high FVIII:C levels are strongly associated with recurrent venous thrombosis, the sample size was powered on FVIII:C (21,22). In a prior study from our group we observed that patients with venous thrombosis have a mean FVIII:C of 141 IU/dL (standard deviation 48) (21). With a number of 2*125=250 participants, we would be able to find a mean (between participants) difference of 17 IU/dL FVIII:C with a 2-sided alpha of 0.05 and 80% power.

Compliance

Adherence to the study protocol was assessed in 2 ways. First, participants who were randomized for rosuvastatin took the first tablet within the presence of an investigator. Second, compliance of rosuvastatin use was monitored by measuring total cholesterol levels at baseline and at end of study in all participants.

Statistical analysis

The general characteristics of the participants are reported as means and ranges. The mean levels with 95% confidence interval (95% CI) of the coagulation factors were calculated at time of randomization and at the end of the study period. Coagulation factors were log-transformed if not normally distributed (this happened to be the case for D-dimer). All analyses were done by intention to treat. Changes of coagulation factors were first expressed as the mean difference (with 95% CI) of coagulation factors at the end of study period in rosuvastatin users versus non-rosuvastatin users. Since we observed that more men were randomized to non-rosuvastatin use and that non-rosuvastatin users were slightly older than those who were randomized to rosuvastatin, we *a-priori* decided to perform both unadjusted and age and sex adjusted analysis by means of linear regression methods to determine the between participant difference of various coagulation factors. Furthermore, we evaluated the change in coagulation factor levels at an individual level. Prespecified exploratory subgroup analyses included the following potential or established prognostic determinants of recurrent venous thrombosis: male/female sex, unprovoked/provoked first event, deep vein thrombosis or pulmonary embolism, and presence or absence of self-reported arterial cardiovascular risk factors (23-25). One post hoc analysis was performed in which we excluded all participants who reported signs or symptoms of an infection during the study, as infections can influence coagulation factors (26).

All analyses were performed with SPSS version 24.0 (IBM, Armonk, NY, USA).

RESULTS

Study population

The START trial was completed as planned; Figure 1 shows the trial profile. A total of 255 participants were randomized between December 2012 and December 2016, with 131 assigned to the rosuvastatin-therapy group, and 124 to the non-rosuvastatin group. As eight participants did not complete the study (5 rosuvastatin users and 3 non-rosuvastatin users), follow-up was thus in 97% complete. The reported reasons for not completing the study are noted in Figure 1. Table 1 presents baseline characteristics. Mean age was 57 years (range 19-82) in rosuvastatin users and 59 years (range 21-81) in non-rosuvastatin users; 68 (54%) of participants allocated to rosuvastatin were men, while this number was 84 (69%) in participants allocated to no treatment. Other reported exposures were balanced at baseline. Rosuvastatin treatment reduced mean cholesterol levels with 1.96 mmol/L (95% CI, 1.83 to 2.09), while this was 0.19 (95% CI, 0.10 to 0.27) in the participants who received no treatment.

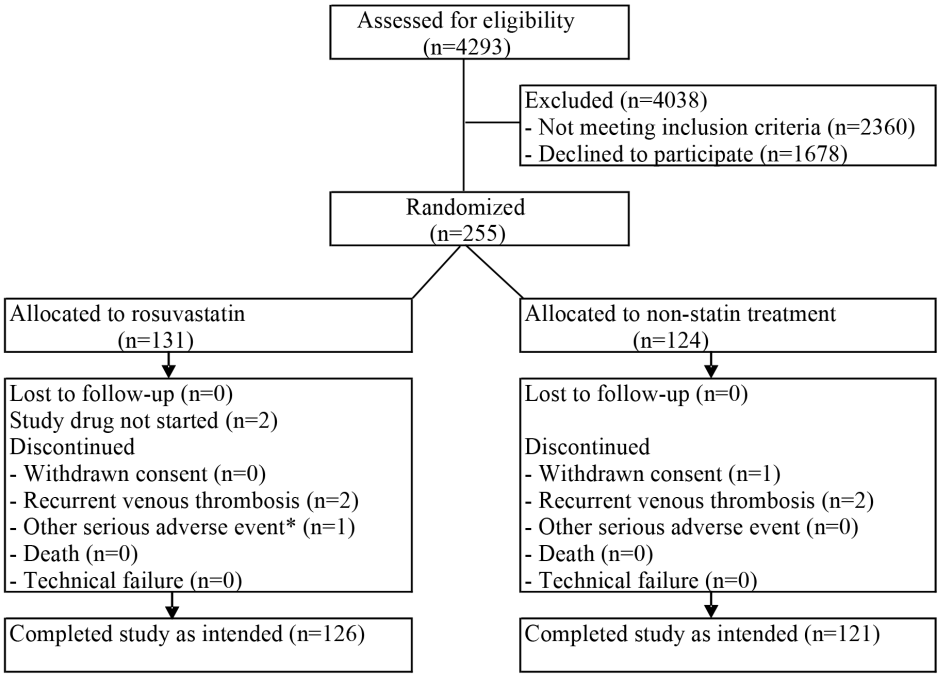


Figure 1. Trial profile

* One participant admitted to hospital with a diagnosis of acute asthma exacerbation

Table 1. Baseline characteristics

	Rosuvastatin users (n=126)		Non-users (n=121)	
General				
Age (years)	57	(19-82)	59	(21-81)
Male	68	(54)	84	(69)
Body mass index (kg/m2)	27.4	(19.2-43.5)	27.7	(17.2-43.2)
Baseline cholesterol (mmol/L)	5.61	(2.95-8.99)	5.59	(3.33-7.89)
Venous thrombosis characteristics				
Type of venous thrombosis				
Deep vein thrombosis	72	(57)	65	(54)
Pulmonary embolism	54	(43)	56	(46)
Unprovoked	57	(45)	64	(53)
Provoked, by	69	(55)	57	(47)
Surgery/ Trauma/ Immobilization	32	(25)	31	(26)
Travel > 4 hrs	22	(18)	14	(12)
Estrogen use (% in women)	24	(41)	14	(38)
Pregnancy/ puerperium (% in women)	0	(0)	2	(5)
Malignancy	2	(2)	8	(7)
Recurrent venous thrombosis	10	(8)	8	(7)
Cardiovascular risk factors				
Current smoking	18	(14)	17	(14)
Hypertension	24	(19)	21	(17)
Diabetes	3	(2)	0	(0)
Overweight	54	(43)	51	(42)
Obesity	29	(23)	35	(29)

Continuous variables denoted as mean (range), categorical variables as number (%).

Outcomes

For all tested coagulation factors in non-users, no difference was observed between the baseline value taken shortly after stopping VKA and the second blood sample drawn after 28 days. In contrast, in rosuvastatin users, coagulation factors decreased after 28 days of treatment compared to baseline. (Figure 2 and Supplementary Table 1). In rosuvastatin users, mean FVIII:C levels were 7.2 IU/dL, (95% CI 2.9 to 11.5) lower, while among non-users, no change in FVIII:C was observed (mean difference -0.1; 95% CI -3.0 to 2.9). The mean difference in FVIII:C change also differed between rosuvastatin users and non-users (-7.2 IU/dL; 95% CI -12.4 to -1.4), with similar results adjusted for age and sex (adjusted mean difference in change (-6.7 IU/dL; 95% CI -12.0 to -1.4). For

FVII:C and FXI:C, results were very similar as for the FVIII:C analyses. VWF:Ag levels did not differ significantly by rosuvastatin use (mean difference -3.1 IU/dL; 95% CI, -9.5 to 3.2 in the paired analysis and -1.4 IU/dl; 95% CI, -8.7 to 6.0 in the between comparison analysis). For natural log-transformed (Ln) D-dimer levels the mean change was 0.01 ng/mL. (95% CI, -0.08 to 0.10) in rosuvastatin users and the between participants change in Ln D-dimer was -0.15 ng/mL (95% CI, -0.32 to 0.01).

In a post hoc analysis, where 8 participants who reported an infection during follow-up were excluded, a reduction of VWF:Ag levels by rosuvastatin use was revealed. In these participants without infection, the mean difference of VWF:Ag level in rosuvastatin users was -7.0 (95% CI -10.8 to -3.2), while among non-users, no change in VWF:Ag was observed. Similarly, the adjusted mean difference in VWF:Ag change differed between rosuvastatin users and non-users (adjusted mean difference in change -5.5 IU/dL (95% CI -10.6 to -0.4). Other coagulation factor outcomes were not materially affected by excluding these 8 participants with infection.

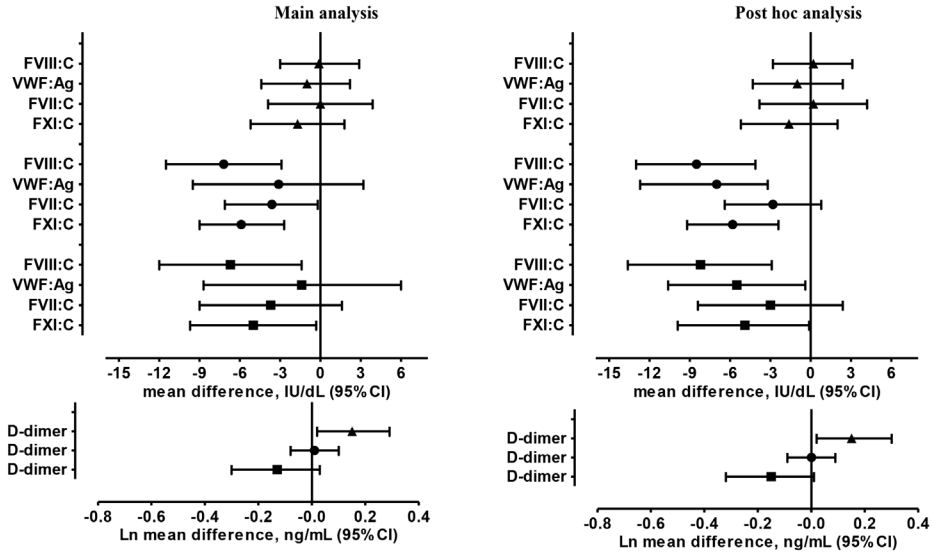


Figure 2. Effects of rosuvastatin on measures of coagulation

In the posthoc analysis, 8 participants who reported an infection at end of study were excluded within person difference in rosuvastatin vs non-users, adjusted for age and sex

▲ between person difference in non-statin users

● between person difference in rosuvastatin users

■ within person difference in rosuvastatin vs non-users, adjusted for age and sex

Subgroup analyses revealed similar results as in the main analysis (Figure 3 for FVIII:C outcomes and Supplementary Table 2 for all measures of coagulation), with the exception of participants with unprovoked venous thrombosis or with cardiovascular risk factors. In these participants the decrease in coagulation factors by rosuvastatin was more pronounced than in those with provoked venous thrombosis or without cardiovascular risk factors, respectively.

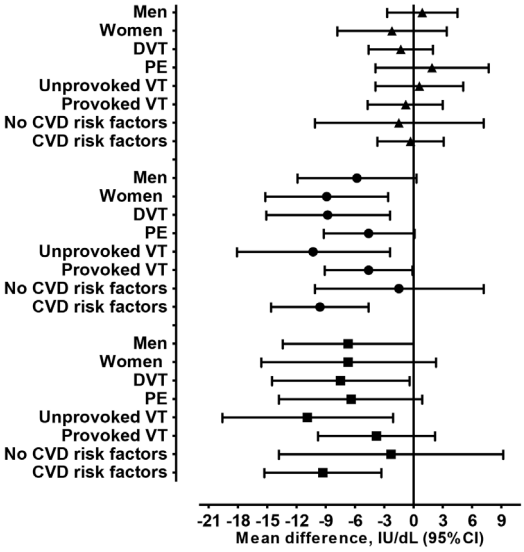


Figure 3. Effects of rosuvastatin on measures of factor VIII:C in prespecified subgroups

▲ between person difference in non-statin users
● between person difference in rosuvastatin users
■ within person difference in rosuvastatin vs non-users
DVT=Deep vein thrombosis, PE=Pulmonary embolism, VT=Venous thrombosis, CVD=Cardiovascular disease

DISCUSSION

This randomized study showed that one month of treatment with rosuvastatin 20 mg/day led to an improved coagulation profile as compared with non-statin users in patients with prior venous thrombosis. Of all tested measures of coagulation, FVIII:C, for which the study was powered, showed the most robust results since rosuvastatin decreased FVIII:C not only within persons, but also between persons and in various subgroups. Other measures of coagulation also showed a shift to a less procoagulant state in participants treated with rosuvastatin, although not always on the level of

statistical significance. Interestingly, the decrease in coagulation factors appeared strongest in participants with unprovoked venous thrombosis and in those with cardiovascular risk factors. This could make sense from a pathophysiological perspective as previous studies showed that patients with unprovoked venous thrombosis more often have endothelial dysfunction or atherosclerosis than patients with provoked venous thrombosis or control individuals (27,28). Statins can modify endothelial function as early as after 28 days of treatment. This is of importance since endothelial dysfunction is associated with a procoagulant state (29,32). Nevertheless, these subgroup analyses must be handled with caution as the study was not designed or powered to analyze differences in subgroups (33).
We observed 2 outliers for VWF:Ag, of which one participant (on rosuvastatin) had an increase of 300 IU/dL at end of study as compared with baseline and another (also on rosuvastatin) had an increase of 103 IU/dL. Both reported an infection at end of study, which may have caused this strong increase in VWF:Ag, and which made us decide to exclude all participants who reported an infection during the study period of 28 days (26). By doing so, VWF:Ag level changes were similar to FVIII:C. This is as expected because VWF is the carrier protein for FVIII in the blood circulation (34). In our study, FXI:C and FVII:C also decreased after treatment with rosuvastatin, which suggests that the anticoagulant activity of rosuvastatin is not only related to measures of coagulation that are affected by endothelial function, but also with liver function (19,20,35). D-dimer levels only showed an effect between participants. For natural log-transformed D-dimer levels, the adjusted mean difference between rosuvastatin users and non-users was -0.13 (95% CI -0.30 to 0.03). Interestingly, this difference was not driven by a lowering of D-dimer levels, but by the absence of an increase in D-dimer in patients using rosuvastatin. Other studies suggest that the initial high risk of recurrent venous thrombosis is due to a rebound phenomenon of several markers of coagulation including D-dimer levels (36-38). Halting an increase in D-dimer by rosuvastatin may therefore be beneficial in patients with previous venous thrombosis in which anticoagulation is withdrawn. Larger studies with longer follow-up (e.g. the Saver trial, registered at www.clinicaltrials.gov as NCT02679664) are however needed to confirm this hypothesis as confidence intervals around the differences in D-dimer levels were wide and included unity.

We showed an overall mean decrease in FVIII:C levels of 7-8 IU/dL in rosuvastatin users, and a 10-12 IU/dL decrease of FVIII:C in participants with unprovoked venous thrombosis. This could be clinically relevant, as every 10 IU/dL decrease in FVIII:C levels is associated with a 15% (95% CI 7% to 22%) decrease in the risk of venous thrombosis (22). In addition, the anticoagulant effect of rosuvastatin was not limited to FVIII:C, but

was shown for several measures of coagulation that are associated with venous thrombosis. This suggests that the reduction of venous thrombosis in the JUPITER trial when using rosuvastatin, and the 25-50% lower risk of recurrent venous thrombosis in observational studies, may be mediated by an improved coagulation profile due to statin use (12, 39-41).

A few issues about this trial warrant comment. First, we noticed a difference in distribution of sex and age after randomization, for which we *a-priori* decided to correct our analysis for these potential confounding factors. These adjustments did not influence our results. Second, previous observational studies and trials consistently showed that, of all statins, rosuvastatin was associated with the strongest risk reduction for venous thrombosis (11-13). For this reason, rosuvastatin 20 mg/day was the statin of choice in START and we cannot rule out that other statins differently or do not affect coagulation. The reason *why* rosuvastatin is able to improve coagulation, is beyond the scope of this study in which we wanted to show that rosuvastatin improves coagulation. It nevertheless may have to do with the fact that of all statins that are currently available on the market, rosuvastatin is most related with halting/regression of atherosclerosis, dyslipidemia, and inflammation (31,42,43). Some studies suggest that dyslipidemia, inflammation, or atherosclerosis, i.e. determinants for arterial cardiovascular disease, also increase the risk of venous thrombosis (27,44,45). The prime mover may therefore be found in one or more of these exposure categories, for which additional studies are warranted.

In order to definitively answer the question whether rosuvastatin should be used for the prevention of recurrent venous thrombosis, an adequately powered randomized clinical trial, with recurrent venous thrombosis as primary end point, should be conducted. The results of this trial support the conduction of such a trial from a pathophysiological perspective.

REFERENCES

1. ISTH Steering Committee for World Thrombosis Day. Thrombosis: a major contributor to the global disease burden. J Thromb Haemost. 2014;12:1580-90.
2. Heit JA. Epidemiology of venous thromboembolism. Nat Rev Cardiol. 2015;12:464-74.3.
3. Kearon C, Akl EA, Ornelas J, Blaivas A, Jimenez D, Bounameaux H, Huisman M, King CS, Morris TA, Sood N, Stevens SM, Vintch JR, Wells P, Woller SC, Moores L. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest. 2016;149:315-52.
4. Lijfering WM, Biedermann JS, Kruip MJ, Leebeek FW, Rosendaal FR, Cannegieter SC. Can we prevent venous thrombosis with statins: an epidemiologic review into mechanism and clinical utility. Expert Rev Hematol. 2016;9:1023-1030.
5. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. Circulation. 2013;127(2):251-7.
6. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, Vittinghoff E, Hulley S. Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The Heart and Estrogen/progestin Replacement Study. Ann Intern Med. 2000;132:689-96.
7. Smeeth L, Douglas I, Hall AJ, Hubbard R, Evans S. Effect of statins on a wide range of health outcomes: a cohort study validated by comparison with randomized trials. Br J Clin Pharmacol. 2009;67(1):99-109.
8. Pai M, Evans NS, Shah SJ, Green D, Cook D, Crowther MA. Statins in the prevention of venous thromboembolism: a meta-analysis of observational studies. Thromb Res. 2011;128(5):422-30.
9. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. Am J Epidemiol. 2003;158:915-20.
10. Danaei G, Tavakkoli M, Hernán MA. Bias in observational studies of prevalent users: lessons for comparative effectiveness research from a meta-analysis of statins. Am J Epidemiol. 2012;175:250-62.
11. Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. BMJ. 2010;340:c2197.
12. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Ridker PM. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009 Apr 30;360(18):1851-61.
13. Rahimi K, Bhala N, Kamphuisen P, Emberson J, Biere-Rafi S, Krane V, Robertson M, Wikstrand J, McMurray J. Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. PLoS Med. 2012;9:e1001310.
14. Adams NB, Lutsey PL, Folsom AR, Herrington DH, Sibley CT, Zakai NA, Ades S, Burke GL, Cushman M. Statin therapy and levels of hemostatic factors in a healthy population: the Multi-Ethnic Study of Atherosclerosis. J Thromb Haemost. 2013;11:1078-84.
15. Sahebkar A, Serban C, Ursoniu S, Mikhailidis DP, Undas A, Lip GY, Bittner V, Ray K, Watts GF, Hovingh GK, Rysz J, Kastelein JJ, Banach M; Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group. The impact of statin therapy on plasma levels of von Willebrand factor antigen. Systematic review and meta-analysis of randomised placebo-controlled trials. Thromb Haemost. 2016;115:520-32.
16. Sahebkar A, Serban C, Mikhailidis DP, Undas A, Lip GY, Muntner P, Bittner V, Ray KK, Watts GF, Hovingh GK, Rysz J, Kastelein JJ, Banach M; Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group. Association between statin use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials. Thromb Haemost. 2015;114:546-57.

17. The Farmacotherapeutisch Kompas, https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/r/rosuvastatine. Accessed May 31, 2017.
18. Undas A, Celinska-Löwenhoff M, Brummel-Ziedins KE, Brozek J, Szczeklik A, Mann KG. Simvastatin given for 3 days can inhibit thrombin generation and activation of factor V and enhance factor Va inactivation in hypercholesterolemic patients. Arterioscler Thromb Vasc Biol. 2005;25:1524-5.
19. Turner NA, Moake JL. Factor VIII Is Synthesized in Human Endothelial Cells, Packaged in Weibel-Palade Bodies and Secreted Bound to ULVWF Strings. PLoS One. 2015;10:e0140740.
20. Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. Blood. 2016;128:2007-2016.
21. Timp JF, Lijfering WM, Flinterman LE, van Hylckama Vlieg A, le Cessie S, Rosendaal FR, Cannegieter SC. Predictive value of factor VIII levels for recurrent venous thrombosis: results from the MEGA follow-up study. J Thromb Haemost. 2015;13:1823-32.
22. Kraaijenhagen RA, in't Anker PS, Koopman MM, Reitsma PH, Prins MH, van den Ende A, Büller HR. High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. Thromb Haemost. 2000;83:5-9.
23. McRae S, Tran H, Schulman S, Ginsberg J, Kearon C. Effect of patient's sex on risk of recurrent venous thromboembolism: a meta-analysis. Lancet. 2006;368:371-8.
24. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet. 2003;362:523-6.
25. Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R, Iotti M, Tormene D, Simioni P, Pagnan A. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica. 2007;92:199-205.
26. Reitsma PH, Branger J, Van Den Blink B, Weijer S, Van Der Poll T, Meijers JC. Procoagulant protein levels are differentially increased during human endotoxemia. J Thromb Haemost. 2003;1:1019-23.
27. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, Prins MH, Girolami A. An association between atherosclerosis and venous thrombosis. N Engl J Med. 2003;348:1435-41.
28. Migliacci R, Becattini C, Pesavento R, Davi G, Vedovati MC, Guglielmini G, Falcinelli E, Ciabattoni G, Dalla Valle F, Prandoni P, Agnelli G, Gresele P. Endothelial dysfunction in patients with spontaneous venous thromboembolism. Haematologica. 2007;92:812-8.
29. Tawakol A, Fayad ZA, Mogg R, Alon A, Klimas MT, Dansky H, Subramanian SS, Abdelbaky A, Rudd JH, Farkouh ME, Nunes IO, Beals CR, Shankar SS. Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study. J Am Coll Cardiol. 2013;62:909-17.
30. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115:1285-95.
31. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM; ASTEROID Investigators.. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA. 2006;295:1556-65.
32. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. N Engl J Med. 2011;364:1746-60.
33. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. Ann Intern Med. 2010;152:726-32.
34. Leebeek FW, Eikenboom JC. Von Willebrand's Disease. N Engl J Med. 2016;375:2067-2080.
35. Wilcox JN, Noguchi S, Casanova J. Extrahepatic synthesis of factor VII in human atherosclerotic vessels. Arterioscler Thromb Vasc Biol. 2003;23:136-41.

36. Cundiff DK. Clinical evidence for rebound hypercoagulability after discontinuing oral anticoagulants for venous thromboembolism. Medscape J Med 2008;10:258.
37. Palareti G, Legnani C, Guazzaloca G, Frascaro M, Grauso F, De Rosa F, Fortunato G, Coccheri S. Activation of blood coagulation after abrupt or stepwise withdrawal of oral anticoagulants – a prospective study. Thromb Haemost 1994;72: 222–6.
38. Martinez C, Katholing A, Folkerts K, Cohen AT. Risk of recurrent venous thromboembolism after discontinuation of vitamin K antagonist treatment: a nested case-control study. J Thromb Haemost. 2016;14:1374-83.
39. Biere-Rafi S, Hutten BA, Squizzato A, Ageno W, Souverein PC, de Boer A, Gerdes VE, Büller HR, Kamphuisen PW. Statin treatment and the risk of recurrent pulmonary embolism. Eur Heart J. 2013;34:1800-6.
40. Schmidt M, Cannegieter SC, Johannesdottir SA, Dekkers OM, Horváth-Puhó E, Sørensen HT. Statin use and venous thromboembolism recurrence: a combined nationwide cohort and nested case-control study. J Thromb Haemost. 2014;12:1207-15.
41. Smith NL, Harrington LB, Blondon M, Wiggins KL, Floyd JS, Sitlani CM, McKnight B, Larson EB, Rosendaal FR, Heckbert SR, Psaty BM. The association of statin therapy with the risk of recurrent venous thrombosis. J Thromb Haemost. 2016;14:1384-92.
42. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ. 2003;326:1423.
43. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, Macfadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ; JUPITER Trial Study Group. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet. 2009;373:1175-82.
44. Horvei LD, Grimnes G, Hindberg K, Mathiesen EB, Njølstad I, Wilsgaard T, Brox J, Braekkan SK, Hansen JB. C-reactive protein, obesity, and the risk of arterial and venous thrombosis. J Thromb Haemost. 2016;14:1561-71.
45. Doggen CJ, Smith NL, Lemaitre RN, Heckbert SR, Rosendaal FR, Psaty BM. Serum lipid levels and the risk of venous thrombosis. Arterioscler Thromb Vasc Biol. 2004;24:1970-5.

Supplementary Table 1. Effects of rosuvastatin on measures of coagulation

			Mean levels				
	Baseline	End of study	Mean* change (95% CI)	Mean difference† in change (95% CI)	Mean difference‡ in change (95% CI)	Mean^ change (95% CI)	Mean difference# in change (95% CI)
Coagulation factors associated with endothelial function							
Factor VIII:C (IU/dL)							
Non users	140	140	-0.1 (-3.0 to 2.9)	Reference	Reference	0.2 (-2.8 to 3.1)	Reference
Rosuvastatin users	146	139	-7.2 (-11.5 to -2.9)	-7.2 (-12.4 to -1.9)	-6.7 (-12.0 to -1.4)	-8.5 (-13.0 to -4.1)	-8.2 (-13.6 to -2.9)
Von Willebrand factor:Ag (IU/dL)							
Non users	165	164	-1.1 (-4.4 to 2.2)	Reference	Reference	-1.0 (-4.3 to 2.4)	Reference
Rosuvastatin users	170	167	-3.1 (-9.5 to 3.2)	-2.1 (-9.3 to 5.1)	-1.4 (-8.7 to 6.0)	-7.0 (-12.7 to -3.2)	-5.5 (-10.6 to -0.4)
Coagulation factors associated with liver function							
Factor VII:C							
Non users	104	104	0.0 (-3.9 to 3.9)	Reference	Reference	0.2 (-3.8 to 4.2)	Reference
Rosuvastatin users	102	98	-3.6 (-7.1 to -0.2)	-3.7 (-8.9 to 1.5)	-3.7 (-9.0 to 1.6)	-2.8 (-6.4 to 0.8)	-3.0 (-8.4 to 2.4)
Factor XI:C							
Non users	124	122	-1.7 (-5.2 to 1.8)	Reference	Reference	-1.6 (-5.2 to 2.0)	Reference
Rosuvastatin users	126	121	-5.9 (-9.0 to -2.7)	-4.2 (-8.8 to 0.5)	-5.0 (-9.7 to -0.3)	-5.8 (-9.2 to -2.4)	-4.9 (-9.9 to -0.1)
Global assay for fibrinolysis ln D-dimer (ng/mL)							
Non users	5.88	6.03	0.15 (0.02 to 0.29)	Reference	Reference	0.15 (0.02 to 0.30)	Reference
Rosuvastatin users	5.89	5.92	0.01 (-0.08 to 0.10)	-0.14 (-0.30 to 0.02)	-0.13 (-0.30 to 0.03)	0.00 (-0.09 to 0.09)	-0.15 (-0.32 to 0.01)

* Paired analysis
† Between comparison analysis
‡ Between comparison analysis, adjusted for age and sex
^ Paired analysis; eight participants who reported an infection at time of end of study excluded
# Between comparison analysis, adjusted for age and sex; 8 participants who reported an infection at time of end of study excluded

Supplementary Table 2. Effects of rosuvastatin on coagulation factors in prespecified subgroups

Factor VIII:C (IU/dL)		Mean levels					
		Baseline	End of study	Mean* change (95% CI)	Mean difference† in change (95% CI)	Mean‡ change (95% CI)	Mean difference^ in change (95% CI)
Sex							
Non users	Men	142	143	0.9 (-2.7 to 4.5)	Reference	0.8 (-2.8 to 4.4)	Reference
Rosuvastatin users	Men	146	140	-5.8 (-11.9 to 0.3)	-6.7 (-13.4 to -0.0)	-6.4 (-12.4 to -0.4)	-7.2 (-13.9 to -0.5)
Non users	Women	137	135	-2.2 (-7.8 to 3.4)	Reference	-1.3 (-6.7 to 4.1)	Reference
Rosuvastatin users	Women	147	138	-8.9 (-15.2 to -2.6)	-6.7 (-15.6 to 2.3)	-10.8 (-17.3 to -4.3)	-9.5 (-18.5 to -0.6)
Type of venous thrombosis							
Non users	DVT	141	140	-1.3 (-4.6 to 2.0)	Reference	-0.9 (-4.2 to 2.3)	Reference
Rosuvastatin users	DVT	149	140	-8.8 (-15.1 to -2.4)	-7.5 (-14.5 to -0.4)	-10.3 (-16.8 to -3.9)	-9.4 (-16.6 to -2.2)
Non users	PE	139	141	1.9 (-3.9 to 7.7)	Reference	1.9 (-3.9 to 7.7)	Reference
Rosuvastatin users	PE	142	137	-4.6 (-9.2 to 0.1)	-6.4 (-13.8 to 0.9)	-5.0 (-9.7 to -0.4)	-6.9 (-14.3 to 0.4)
Venous thrombosis risk factors							
Non users	Unprovoked	144	144	0.6 (-3.9 to 5.1)	Reference	0.5 (-4.1 to 5.1)	Reference
Rosuvastatin users	Unprovoked	153	143	-10.3 (18.1 to -2.4)	-10.9(-19.6 to -2.1)	-11.6 (-19.9 to -3.4)	-12.2 (-21.1 to -3.2)
Non users	Provoked	137	136	-0.8 (-4.7 to 3.0)	Reference	-0.2 (-3.9 to 3.5)	Reference
Rosuvastatin users	Provoked	141	136	-4.6 (-9.1 to -0.1)	-3.8 (-9.8 to 2.2)	-5.7 (-10.0 to -1.3)	-5.5 (-11.1 to 0.2)
Cardiovascular risk factors#							
Non users	Absent	135	136	0.8 (-5.8 to 7.4)	Reference	0.8 (-5.8 to 7.4)	Reference
Rosuvastatin users	Absent	143	141	-1.5 (-10.1 to 7.2)	-2.3 (-13.8 to 9.2)	-1.8 (-10.9 to 7.3)	-2.6 (-14.4 to 9.1)
Non users	Present	142	142	-0.3 (-3.7 to 3.1)	Reference	0.0 (-3.4 to 3.4)	Reference
Rosuvastatin users	Present	148	138	-9.6 (-14.6 to -4.6)	-9.3 (-15.3 to -3.3)	-11.1 (-16.0 to -6.0)	-11.1 (-16.9 to -5.2)

* Paired analysis
† Between comparison analysis
‡ Paired analysis, 8 participants who reported an infection at time of end of study excluded
^ Between comparison analysis, 8 participants who reported an infection at time of end of study excluded
# Defined as self-reported current smoking, hypertension, diabetes mellitus, or a body mass index > 25 kg/m^2

Supplementary Table 2. ***Continued***

Factor VII:C (IU/dL)		Mean levels					
		Baseline	**End of study**	**Mean* change (95% CI)**	**Mean difference† in change (95% CI)**	**Mean‡ change (95% CI)**	**Mean difference^ in change (95% CI)**
Sex							
Non users	Men	104	103	-0.8 (-5.8 to 4.3)	Reference	-0.8 (-5.9 to 4.3)	Reference
Rosuvastatin users	Men	95	93	-2.7 (-7.7 to 2.3)	-2.0 (-9.1 to 5.2)	-2.0 (-6.9 to 2.8)	-1.2 (-8.3 to 5.9)
Non users	Women	103	105	1.9 (-4.0 to 7.8)	Reference	2.0 (-4.1 to 8.1)	Reference
Rosuvastatin users	Women	109	105	-4.7 (-9.6 to 0.2)	-6.6 (-14.2 to 1.0)	-3.8 (-9.0 to 1.3)	-5.8 (-13.7 to 2.1)
Type of venous thrombosis							
Non users	DVT	104	104	-0.5 (-5.8 to 4.8)	Reference	-0.5 (-6.0 to 5.0)	Reference
Rosuvastatin users	DVT	102	98	-4.4 (-9.0 to 0.3)	-3.9 (-10.9 to 3.2)	-3.4 (-8.2 to 1.3)	-2.9 (-10.1 to 4.3)
Non users	PE	103	104	0.9 (-4.8 to 6.6)	Reference	0.9 (-4.8 to 6.6)	Reference
Rosuvastatin users	PE	101	98	-2.4 (-7.5 to 2.7)	-3.3 (-10.8 to 4.3)	-1.8 (-6.9 to 3.3)	-2.7 (-10.3 to 4.9)
Venous thrombosis risk factors							
Non users	Unprovoked	99	101	2.2 (-3.9 to 8.3)	Reference	2.2 (-4.0 to 8.4)	Reference
Rosuvastatin users	Unprovoked	97	95	-1.8 (-7.1 to 3.5)	-4.0 (-12.1 to 4.0)	-0.2 (-5.4 to 5.1)	-2.4 (-10.6 to 5.8)
Non users	Provoked	109	106	-2.5 (-7.2 to 2.2)	Reference	-2.5 (-7.3 to 2.3)	Reference
Rosuvastatin users	Provoked	106	101	-5.2 (-9.9 to -0.5)	-2.7 (-9.3 to 3.9)	-5.0 (-9.8 to -0.3)	-2.6 (-9.3 to 4.1)
Cardiovascular risk factors#							
Non users	Absent	99	100	0.2 (-6.1 to 6.2)	Reference	0.2 (-6.1 to 6.5)	Reference
Rosuvastatin users	Absent	107	102	-5.1 (-10.0 to -0.3)	-5.3 (-13.0 to 2.3)	-4.5 (-9.4 to 0.5)	-4.7 (-12.4 to 3.0)
Non users	Present	105	105	0.0 (-4.7 to 4.7)	Reference	0.0 (-4.8 to 4.8)	Reference
Rosuvastatin users	Present	100	97	-3.0 (-7.5 to 1.5)	-3.0 (-9.5 to 3.5)	-2.2 (-6.7 to 2.4)	-2.2 (-8.8 to 4.4)

* Paired analysis
† Between comparison analysis
‡ Paired analysis, 8 participants who reported an infection at time of end of study excluded
^ Between comparison analysis, 8 participants who reported an infection at time of end of study excluded
# Defined as self-reported current smoking, hypertension, diabetes mellitus, or a body mass index > 25 kg/m^2

Supplementary Table 2. ***Continued***

Factor XI:C (IU/dL)		Mean levels					
		Baseline	**End of study**	**Mean* change (95% CI)**	**Mean difference† in change (95% CI)**	**Mean‡ change (95% CI)**	**Mean difference^ in change (95% CI)**
Sex							
Non users	Men	121	117	-4.5 (-7.4 to -1.6)	Reference	-4.4 (-7.4 to -1.5)	Reference
Rosuvastatin users	Men	123	116	-7.0 (-9.4 to -4.5)	-2.5 (-6.4 to 1.3)	-7.1 (-9.5 to -4.6)	-2.6 (-6.5 to 1.3)
Non users	Women	129	134	4.5 (-4.8 to 13.9)	Reference	5.0 (-4.5 to 14.6)	Reference
Rosuvastatin users	Women	130	125	-4.6 (-10.9 to 1.8)	-9.1 (-19.8 to 1.6)	-4.3 (-11.2 to 2.7)	-9.3 (-20.6 to 2.0)
Type of venous thrombosis							
Non users	DVT	122	120	-2.1 (-5.8 to 1.7)	Reference	-1.8 (-5.7 to 2.0)	Reference
Rosuvastatin users	DVT	125	119	-5.8 (-10.6 to -1.0)	-3.7 (-9.8 to 2.4)	-5.7 (-10.8 to -0.6)	-3.9 (-10.3 to 2.5)
Non users	PE	126	125	-1.1 (-8.1 to 5.8)	Reference	-1.1 (-8.1 to 5.8)	Reference
Rosuvastatin users	PE	129	123	-6.1 (-8.6 to -3.6)	-4.9 (-12.2 to 2.4)	-6.0 (-8.6 to -3.5)	-4.9 (-12.3 to 2.5)
Venous thrombosis risk factors							
Non users	Unprovoked	123	121	-2.1 (-7.6 to 3.4)	Reference	-2.0 (-7.6 to 3.6)	Reference
Rosuvastatin users	Unprovoked	126	118	-7.4 (-13.2 to -1.6)	-5.3 (-13.2 to 2.7)	-7.4 (-13.6 to -1.1)	-5.3 (-13.6 to 3.0)
Non users	Provoked	125	123	-1.2 (-5.4 to 2.9)	Reference	-1.0 (-5.2 to 3.2)	Reference
Rosuvastatin users	Provoked	127	123	-4.6 (-7.8 to -1.4)	-3.3 (-8.5 to 1.8)	-4.6 (-7.9 to -1.3)	-3.6 (-8.8 to 1.7)
Cardiovascular risk factors#							
Non users	Absent	122	116	-6.0 (-12.8 to 0.7)	Reference	-6.0 (-12.8 to 0.7)	Reference
Rosuvastatin users	Absent	124	121	-2.9 (-6.8 to 1.0)	3.1 (-4.0 to 10.2)	-2.6 (-6.7 to 1.5)	3.4 (-3.9 to 10.7)
Non users	Present	124	124	-0.5 (-4.6 to 3.5)	Reference	-0.3 (-4.5 to 3.8)	Reference
Rosuvastatin users	Present	127	120	-7.1 (-11.3 to -2.9)	-6.6 (-12.4 to -0.8)	-7.1 (-11.5 to -2.8)	-6.8 (-12.8 to -0.9)

† Between comparison analysis
‡ Paired analysis, 8 participants who reported an infection at time of end of study excluded
^ Between comparison analysis, 8 participants who reported an infection at time of end of study excluded
# Defined as self-reported current smoking, hypertension, diabetes mellitus, or a body mass index > 25 kg/m^2
* Paired analysis

Supplementary Table 2. ***Continued***

Ln D-dimer (ng/ml)		Mean levels					
		Baseline	End of study	Mean* change (95% CI)	Mean difference† in change (95% CI)	Mean‡ change (95% CI)	Mean difference^ in change (95% CI)
Sex							
Non users	Men	5.93	6.13	0.20 (0.04 to 0.36)	Reference	0.20 (0.05 to 0.37)	Reference
Rosuvastatin users	Men	5.83	5.80	-0.03 (-0.17 to 0.10)	-0.23 (-0.44 to -0.02)	-0.03 (-0.17 to 0.10)	-0.24 (-0.46 to -0.03)
Non users	Women	5.77	5.80	0.03 (-0.23 to 0.30)	Reference	0.05 (-0.22 to 0.31)	Reference
Rosuvastatin users	Women	5.99	6.05	0.06 (-0.05 to 0.18)	0.03 (-0.22 to 0.28)	0.05 (-0.07 to 0.17)	0.00 (-0.29 to 0.30)
Type of venous thrombosis							
Non users	DVT	5.82	6.00	0.18 (-0.01 to 0.37)	Reference	0.20 (0.01 to 0.39)	Reference
Rosuvastatin users	DVT	5.89	5.95	0.06 (-0.06 to 0.18)	-0.12 (-0.34 to 0.10)	0.06 (-0.07 to 0.18)	-0.14 (-0.37 to 0.08)
Non users	PE	5.97	6.07	0.10 (-0.09 to 0.29)	Reference	0.10 (-0.09 to 0.29)	Reference
Rosuvastatin users	PE	5.94	5.87	-0.07 (-0.20 to 0.06)	-0.17 (-0.40 to 0.05)	-0.08 (-0.21 to 0.05)	-0.18 (-0.41 to 0.04)
Venous thrombosis risk factors							
Non users	Unprovoked	5.96	6.16	0.20 (0.05 to 0.34)	Reference	0.20 (0.06 to 0.35)	Reference
Rosuvastatin users	Unprovoked	5.86	5.90	0.04 (-0.11 to 0.19)	-0.16 (-0.36 to 0.05)	0.03 (-0.13 to 0.18)	-0.18 (-0.39 to 0.03)
Non users	Provoked	5.78	5.88	0.10 (-0.15 to 0.34)	Reference	0.10 (-0.14 to 0.36)	Reference
Rosuvastatin users	Provoked	5.94	5.93	-0.01 (-0.12 to 0.10)	-0.11 (-0.36 to 0.14)	-0.01 (-0.13 to 0.10)	-0.12 (-0.38 to 0.13)
Cardiovascular risk factors#							
Non users	Absent	5.86	6.01	0.15 (-0.15 to 0.46)	Reference	0.15 (-0.15 to 0.46)	Reference
Rosuvastatin users	Absent	5.81	5.85	0.04 (-0.17 to 0.26)	-0.11 (-0.46 to 0.24)	0.04 (-0.19 to 0.26)	-0.12 (-0.48 to 0.24)
Non users	Present	5.88	6.03	0.15 (0.0 to 0.30)	Reference	0.16 (0.01 to 0.32)	Reference
Rosuvastatin users	Present	5.94	5.94	0.00 (-0.10 to 0.10)	-0.15 (-0.33 to 0.03)	-0.01 (-0.11 to 0.09)	-0.17 (-0.36 to 0.02)

* Paired analysis
† Between comparison analysis
‡ Paired analysis, 8 participants who reported an infection at time of end of study excluded
^ Between comparison analysis, 8 participants who reported an infection at time of end of study excluded
# Defined as self-reported current smoking, hypertension, diabetes mellitus, or a body mass index > 25 kg/m^2

Supplementary Table 2. ***Continued***

Von Willebrand Factor: Ag (IU/dL)		Mean levels					
		Baseline	**End of study**	**Mean* change (95% CI)**	**Mean difference† in change (95% CI)**	**Mean‡ change (95% CI)**	**Mean difference^ in change (95% CI)**
Sex							
Non users	Men	171	172	1.0 (-3.0 to 5.0)	Reference	1.2 (-2.8 to 5.2)	Reference
Rosuvastatin users	Men	170	168	-2.2 (-12.6 to 8.1)	-3.3 (-13.5 to 6.9)	-6.9 (-11.6 to -2.2)	-8.1 (-14.2 to -2.0)
Non users	Women	153	147	-5.7 (-11.5 to 0.1)	Reference	-5.9 (-11.9 to 0.03)	Reference
Rosuvastatin users	Women	169	165	-4.2 (-11.4 to 3.1)	1.5 (-7.6 to 10.7)	-7.2 (-13.6 to -0.7)	-1.2 (-9.9 to 7.4)
Type of venous thrombosis							
Non users	DVT	167	165	-1.9 (-6.4 to 2.7)	Reference	-1.8 (-6.4 to 2.9)	Reference
Rosuvastatin users	DVT	174	172	-2.3 (-11.8 to 7.2)	-0.4 (-11.1 to 10.3)	-7.8 (-12.9 to -2.8)	-6.1 (-12.9 to 0.7)
Non users	PE	163	163	0.2 (-4.5 to 4.9)	Reference	0.2 (-4.5 to 4.9)	Reference
Rosuvastatin users	PE	162	157	-4.7 (-10.9 to 1.5)	-4.9 (-12.6 to 2.8)	-5.6 (-11.7 to 0.4)	-5.8 (-13.4 to 1.7)
Venous thrombosis risk factors							
Non users	Unprovoked	169	169	0.5 (-4.0 to 5.0)	Reference	0.7 (-3.8 to 5.3)	Reference
Rosuvastatin users	Unprovoked	179	174	-4.9 (-16.6 to 6.7)	-5.5 (-17.3 to 6.4)	-10.9 (-15.2 to -6.7)	-11.7 (-17.9 to -5.4)
Non users	Provoked	161	158	-3.0 (-7.9 to 2.0)	Reference	-3.1 (-8.1 to 2.0)	Reference
Rosuvastatin users	Provoked	162	160	-1.6 (-8.4 to 5.1)	1.3 (-7.3 to 10.0)	-3.8 (-9.8 to 2.2)	-0.7 (-8.5 to 7.0)
Cardiovascular risk factors#							
Non users	Absent	161	161	-0.6 (-9.2 to 8.1)	Reference	-0.6 (-9.2 to 8.1)	Reference
Rosuvastatin users	Absent	166	161	-5.3 (-13.4 to 2.8)	-4.8 (-16.5 to 7.0)	-6.2 (-14.4 to 1.9)	-5.7 (-17.4 to 6.0)
Non users	Present	166	165	-1.2 (-4.8 to 2.4)	Reference	-1.1 (-4.7 to 2.5)	Reference
Rosuvastatin users	Present	171	169	-2.3 (-10.7 to 6.1)	-1.1 (-10.2 to 8.0)	-7.3 (-11.7 to -2.9)	-6.2 (-11.8 to -0.6)

* Paired analysis
† Between comparison analysis
‡ Paired analysis, 8 participants who reported an infection at time of end of study excluded
^ Between comparison analysis, 8 participants who reported an infection at time of end of study excluded
# Defined as self-reported current smoking, hypertension, diabetes mellitus, or a body mass index > 25 kg/m^2

CHAPTER 13

General Discussion

Patients suffering from venous thrombosis or patients with atrial fibrillation, who are at risk of thrombotic complications, require anticoagulant therapy to prevent progression or onset of thrombotic events. Several anticoagulant agents are currently available, registered for different thrombotic indications. Of these, atrial fibrillation, venous thromboembolism and mechanical heart valves are the most common. Anticoagulant therapy is initiated in these patients to reduce coagulation capacity (thrombin generation). However, due to this change in the hemostatic balance, treatment with anticoagulants is accompanied by an increased risk of bleeding. Therefore, individual assessment of the risk of thrombotic versus bleeding complications is warranted before initiating anticoagulant therapy. Furthermore, during treatment with anticoagulants, patients should be managed adequately to continuously balance the risk of thrombotic and bleeding complications. Due to the relatively high bleeding risk associated with currently available anticoagulants, ongoing research focuses on development of safer anticoagulant strategies with similar efficacy.

The aim of this thesis was to study current monitoring and management of anticoagulation therapy, especially therapy with vitamin K antagonists (VKA), in order to further improve the efficacy and safety of anticoagulant therapy in the future. Therefore, we first investigated the accuracy and safety of point-of-care INR monitoring, using the CoaguChek XS system, as a more patient-friendly alternative to laboratory INR monitoring in patients on VKA. We also evaluated the sensitivity of different thromboplastins, including the one incorporated in the CoaguChek XS test strips, regarding sensitivity to clotting factor VII, an important determinant of INR stability during VKA therapy. Next, we performed studies focusing on current VKA management. We evaluated the bleeding risk associated with the use of different low-molecular-weight heparin (LMWH) agents during combined treatment with VKA in a large cohort of patients with new onset venous thrombosis. Furthermore, we investigated the agreement between two commonly used methods to estimate the quality of anticoagulation control during VKA therapy and we identified predictors of oral cavity bleeding and evaluated clinical outcome after dental procedures in patients on VKA. Finally, we investigated anticoagulant effects of statin therapy in patients with a history of venous thrombosis to explore the potential use of statins as a safer strategy to prevent recurrent venous thromboembolism.

Point-of-care monitoring in patients on vitamin K antagonists

In this thesis we have shown, in chapter 2, in a study cohort of nearly 3000 patients on VKA, that point-of-care (POC) INR results from the CoaguChek XS correlate strongly with the laboratory STA-R Evolution (Hepato Quick) INR results, especially in the

therapeutic INR range. This is in line with previous smaller studies comparing this POC device with different laboratory methods (1-6). Our findings implicate that INR results from the CoaguChek XS are interchangeable with different currently used laboratory methods. In daily practice a POC test is mainly used by self-monitoring patients, but our study indicates that VKA dosing schedules do not need adjustment to POC results. Therefore, thrombosis services can relatively easy implement this method for routine INR measurement in patients unwilling or incapable to perform INR self-monitoring (e.g. non-self-monitoring patients). In our study we did find some INR discrepancies between Coaguchek XS and STA-R Evolution (Hepato Quick) INR results (1). We observed that the CoaguChek XS slightly underestimated the INR at subtherapeutic levels (INR<2.0) and increasingly overestimated the INR at supratherapeutic levels (INR>4.5) compared to this laboratory method, a pattern that has been previously reported in literature (1, 7-9). It is important to mention though that the INR is, strictly speaking, only valid up until an INR of 4.5, since only plasma samples with an INR between 1.5-4.5 are used during the calibration process of any reagent-instrument combination used for INR measurement (10). Furthermore, similar INR discrepancy patterns have also been reported between different validated laboratory methods, including in comparisons where the same analyzer was used in combination with thromboplastin reagents from different sources (e.g. recombinant vs. tissue-derived) (11,12). Thus, it is likely that the INR differences are mostly thromboplastin reagent dependent rather than instrument dependent (12).

Apart from satisfactory accuracy, measurement methods should also be precise enough in terms of reproducibility. In chapter 3 we have shown that the CoaguChek XS is precise enough for INR monitoring of both self-monitoring and non-self-monitoring patients (e.g. INR measured by a clinic) in relation to the biological variation of the INR during VKA treatment (13). The biological INR variation is based on the total within-subject INR variation during stabile treatment, corrected for the analytical imprecision of the used measurement method (14). The analytical imprecision, defined as the imprecision coefficient of variation, should, preferably, not exceed 50% of the biological variation of the analyte (15, 16). The reported imprecision coefficient of the CoaguChek XS in high quality studies is approximately 2-4%, which is similar to the 2.9% we observed in our own study (1,2,7,9). The biological INR variation in both self-monitoring and non-self-monitoring patients on acenocoumarol (approximately 10%) or phenprocoumon (approximately 8%) we found in our study was therefore in agreement with these goals. This implies that CoaguChek XS INR values are not only accurate, but also that the CoaguChek XS system is precise enough for clinical use in terms of reproducibility. Furthermore, the biological INR variation we observed during

CoaguChek XS monitoring of non-self-monitoring patients was comparable with the biological INR variation as observed in a previous study in laboratory monitored patients on warfarin (17). This suggests that our findings regarding biological variation in non-self-monitoring patients are likely to be also valid for patients on warfarin, enhancing the generalizability of our findings. Importantly, we have shown in chapter 4 that, apart from satisfactory analytical performance, POC INR monitoring using the CoaguChek XS by a thrombosis service is a safe and effective alternative for laboratory INR monitoring in non-self-monitoring patients in terms of clinical outcome (e.g. thrombotic and bleeding complications) (18). This is highly relevant since POC INR measurement requires far less logistics than laboratory INR measurement for which a venous blood sample needs to be drawn, transported and processed. In addition, the POC INR result is immediately available making it possible to adjust VKA dose more rapidly. Furthermore, previous studies have shown that POC monitoring, either community based or through self-monitoring or self-management, is associated with increased patient satisfaction and experienced quality of life compared to routine monitoring (19-22). Therefore, POC monitoring in non-self-monitoring patients can improve the quality of life and eliminate the burden of venous blood sampling in the vast majority of patients currently on VKA. Finally, since patients become familiar with this method for INR measurement, more patients may be tempted to consider INR self-monitoring or even INR self-management due to their repeated experiences with the device. This may potentially further improve quality of care in these patients, since INR self-monitoring and self-management are associated with better anticoagulation control and a decreased risk of thrombotic complications (23).

Some remarks should be made. First, several other POC devices have become available for INR monitoring. The results of this thesis cannot be directly translated to all situations where a different POC device is used, since suboptimal accuracy of some of these devices has been reported (24). Reliable INR results are pivotal to determine the need for dose adjustment in patients on VKA, which directly influences anticoagulation control and therefore the risk of complications during treatment. Thus, it is important that the accuracy and adequacy of these devices, including the used test strips, are continuously and systematically monitored. External quality assessment (EQA) programs for POC devices have been established and these programs contribute to the continuous safe use of these devices (25). Summarizing, the above-mentioned findings suggest that the use of this more patient-friendly monitoring method is suitable for virtually all patients on VKA, if adequate quality control of the POC device and test strips is established. However, POC monitoring in one specific group of patients should be discussed. The accuracy of POC INR results, including CoaguChek XS results,

has been questioned in patients with the antiphospholipid syndrome (APS). This has been triggered by reports of significant INR discrepancies between POC devices and laboratory assays in APS patients, although conflicting results on this subject have also been reported (26-28).

It is good to emphasize though that similar INR discrepancies are also present when comparing different laboratory assays in APS patients, especially when results are obtained with thromboplastins from different sources (e.g. recombinant vs. tissue-derived) (29). One study comparing INR results of different assays amongst each other, including the CoaguChek XS, in APS-patients and APL-negative controls, showed that the agreement between CoaguChek XS results and laboratory methods was dependent on the used thromboplastin in the laboratory assay (30). Agreement between CoaguChek XS results and a laboratory method was better when a recombinant thromboplastin (Innovin), similar to the one incorporated in the CoaguChek XS test strip, was used (30). Irrespective of the assay comparison, it is good to note that the "true" INR that best reflects the anticoagulant state of any patient, including those with APS, is unknown. To date, no studies have been conducted relating different monitoring methods to clinical outcome in APS patients. Although we found no differences regarding clinical outcome in unselected patients after switching from a tissue-extract thromboplastin (Hepato Quick) to a recombinant thromboplastin (CoaguChek XS) monitoring, we cannot definitively exclude such an effect in patients with APS (18). Currently, the subcommittee on anticoagulation control of the International Society of Thrombosis and Haemostasis advises to be cautious when interpreting INR results from POC devices in patients with APS and to validate the accuracy of POC readings before systematic use in these patients (31). Future studies should investigate the impact of POC monitoring on treatment quality and clinical outcome in patients with the anti-phospholipid syndrome.

Reagent-dependent differences in INR stability due to differences in FVII sensitivity

Despite the safety of POC INR monitoring, we found that anticoagulation control, defined as percentage of time spent within the therapeutic range (TTR%), was statistically significantly poorer during POC monitoring as compared with standard laboratory monitoring (Chapter 4) (18). It should be emphasized that despite the slightly lower quality of anticoagulation control during POC monitoring, treatment quality remained satisfactory in the cohort of patients we studied, as reflected by high mean individual TTR results during POC monitoring and similar patient outcomes as during laboratory monitoring (18). Therefore, the question is whether the statistically

significant difference in TTR we observed in our study is of any clinical importance. It is likely that the lower TTR during POC monitoring we observed in our study can be explained by the difference in thromboplastin reagent incorporated in the test strips of the CoaguChek XS (recombinant human), compared to the reagent used in the laboratory method (tissue-derived). Similar differences in TTR have been observed between patients monitored with laboratory methods using thromboplastins from different sources (32). The fact that we found no thromboplastin-dependent difference in quality of anticoagulation control in patients on phenprocoumon, was a strong indicator that differences in FVII sensitivity between reagents might explain this difference, since FVII levels fluctuate far less in patients on phenprocoumon than in patients on acenocoumarol (14,18,33). Previous studies have suggested that FVII fluctuations can, to a large extent, explain the variability of the INR in patients treated with warfarin and acenocoumarol (34). Therefore, reagent-dependent differences in FVII-sensitivity would explain the negative impact of POC monitoring on TTR in patients on acenocoumarol, while not affecting TTR in patients on phenprocoumon, when switching from a tissue-derived thromboplastin to a recombinant thromboplastin, as we observed in our study (18). Although differences in FVII-sensitivity have been clearly demonstrated between non-commercial thromboplastins, this has not been the case for different commercial thromboplastins used for patient monitoring in daily practice (35). In chapter 5, we demonstrated that differences in FVII-sensitivity also exist between commercially used thromboplastins, even despite optimal calibration (36). This implicates that FVII-mediated INR stability is affected both by the type of VKA used by the patient, as well as the reagent incorporated in the method used for monitoring. These insights obtained from our studies can be used to minimize FVII-mediated INR fluctuations and their impact on anticoagulation control during VKA treatment.

Strategies to negate FVII-mediated INR fluctuations

Recently, a new thromboplastin reagent for laboratory INR measurement has been introduced, completely insensitive to FVII, and thus also to FVII-mediated INR fluctuations (37). This reagent, the Fiix-PT, has shown to be non-inferior to routine laboratory monitoring regarding clinical outcome, both in terms of efficacy (thrombotic risk) and safety (bleeding risk), but improved INR stability and reduced the number of required dose adjustments in patients on warfarin (38). Since INR stability is related to the monitoring frequency, also the average required monitoring frequency in these patients was reduced (38). Furthermore, Fiix-PT was associated with a shorter time until stable VKA dose in warfarin naïve patients (38, 39). It would be interesting to explore whether this reagent can be incorporated in a POC device, to improve INR stability and reduce the measure frequency during POC monitoring.

A different strategy to improve INR stability and anticoagulation control would be to reduce the occurrence and magnitude of FVII fluctuations in patients on VKA. FVII fluctuations are strongly dependent of the half-life of the used VKA agent. Medium to large fluctuations have been observed in acenocoumarol and warfarin treated patients, while in patients on the long-acting phenprocoumon FVII levels fluctuate far less during stabile treatment (33, 40). Therefore, switching patients from acenocoumarol and warfarin to phenprocoumon may improve INR stability, especially benefiting patients monitored with recombinant thromboplastins (32). In general, clinicians should be aware that the combination of thromboplastin reagent used for monitoring, and the half-life of the used VKA by their patients, affects the average quality of anticoagulation control in their patient population. Regarding this, it should also be mentioned that switching unselected patients from a tissue-derived thromboplastin (Hepato Quick) to a human recombinant reagent (CoaguChek XS) was safe and not associated with poorer clinical outcome in our study despite lower TTRs (18). Similarly, despite consistently higher TTR and less INR variability as observed during phenprocoumon treatment, the risk of bleeding and thrombotic complication is equal or even lower in patients treated with acenocoumarol than in patients treated with phenprocoumon (41, 42). Large cohort studies, following these patients over a longer period, may be needed to definitively exclude whether these small FVII-mediated differences in TTR do affect long-term clinical outcome.

It has also been postulated that TTR and INR stability could be improved by routine supplementation of fixed low doses of vitamin K, to decrease dietary-induced FVII fluctuations (43). Several studies on this topic have been performed using different vitamin K doses, mostly showing minor or no improvement in TTR (44-49). Furthermore, these studies also failed to demonstrate lower rates of bleeding or thrombotic complications, although most of these studies were underpowered for clinical outcomes. Therefore, routine supplementation of fixed dose vitamin K is currently not recommended based on the available evidence (48, 50).

Lastly, INR stability can also be influenced by cytochrome P450-mediated drug and food interactions. Recently, a new VKA agent that is metabolized by esterases, tecarfarin, has been tested in a randomized controlled trial in patients on warfarin (51). However, this new VKA was not associated with higher TTR compared to warfarin, or with a lower risk of thrombotic or bleeding complications (51). Nevertheless, in general, reducing the number of food and drug interactions of VKA may improve long-term clinical outcome and can reduce the required monitoring frequency in these patients.

Management of VKA therapy

In the HARM study, van den Bemt et al. identified oral anticoagulants as one of the most important sources of preventable hospital admissions related to medications (52). Due to the many food and drug interactions of VKA, and their unpredictable anticoagulant effect, these drugs require monitoring and constant management to reduce the risk of complications during exposure. Also, it is well known that patients on anticoagulants are at increased risk of thrombotic and bleeding complications in the perioperative period. Therefore, standardized management strategies, incorporating both the risk of the procedure, as well as the thrombotic and bleeding risk of the individual patient, have been proposed (53,54). In order to reduce periprocedural complications, we evaluated the perioperative management strategy and identified several predictors of bleeding after low-risk and high-risk dental procedures (Chapter 6). We found that most predictors of postoperative bleeding were specifically related to the followed perioperative management strategy and that the risk of bleeding increased when guideline recommendations were not followed. This underlines the importance of adherence to management guidelines to optimize clinical outcome in VKA treated patients undergoing invasive procedures. In general, perioperative management guidelines might be able to be refined through further risk-stratification of specific procedures. This is in line with current developments in the field, where specific low-risk procedures such as colonoscopies and pacemaker implantations are now being performed at therapeutic INR levels (54,55). To achieve this, information exchange between hospitals and anticoagulation clinics would also need further improvement and standardization. Currently, a study is being conducted investigating whether in-hospital antithrombotic stewardship can improve the efficacy and safety of antithrombotic therapy during and after hospitalization (56). The outcomes of this study may also improve future VKA management immediately after hospitalization, since patients are also at an increased bleeding risk during the first three months of VKA therapy (57). Part of this increased risk can be explained by the concomitant usage of low-molecular-weight heparin (LMWH) therapy, which is continued until an adequate INR is reached. These patients are therefore shortly exposed to dual anticoagulant therapy. Moreover, due to standard VKA startup dosing schedules, some patients already present themselves with supratherapeutic INR levels at anticoagulation clinics before LMWH treatment is discontinued, also associated with an increased bleeding risk (57). We evaluated the bleeding risk during combined LMWH and VKA therapy in patients with acute venous thrombosis, and investigated whether differences between LMWH agents existed regarding the risk of bleeding. (Chapter 6). It was reassuring that the absolute bleeding risk during combined VKA and LMWH therapy was low, and not dependent on the used LMWH agent. Our findings suggest

that twice daily LMWH dosing is associated with an increased bleeding risk, which was also recently reported in patients initiating VKA treatment and enoxaparin for acute venous thrombosis (58). This was a propensity-score matched study, making it less likely that confounding could explain the increased bleeding risk of twice daily LMWH dosing compared to once daily dosing in these patients, although residual confounding by indication can never be completely excluded in non-randomized studies. Together though, these two studies strengthen the observation of two meta-analyses reporting a trend towards an increased bleeding risk associated with twice daily LMWH dosing (59,60). An adequately powered randomized controlled trial may be needed to confirm or exclude this definitively.

Finally, we compared two different methods (the cross-sectional proportion (CSP) of INR results within therapeutic range and the time in therapeutic range (TTR) according to Rosendaal) to assess therapeutic quality control in several different groups of patient on VKA (61). The TTR is calculated based on linear interpolation between consecutive INR measurements, while the CSP is defined as the percentage of patients for whom their last INR result was within the therapeutic range (61). Both methods have their advantages and disadvantages, but both are suitable to estimate the average quality of anticoagulation control of a group of patients, which can be used as a marker of quality of care by health care providers. In almost all comparisons, both methods showed nearly identical results, suggesting that the results of both methods can be used interchangeably (61). This is convenient since the Rosendaal method is not always available, especially in low-resource countries, which can use the CSP method as an alternative to estimate the quality of care they provide. An important limitation of the CSP method is that it cannot be used for individual risk assessment of thrombotic and bleeding complications, since it provides no information on individual longitudinal anticoagulation control (62). Several other parameters of anticoagulation control have been linked to clinical outcome during VKA therapy in individual patients, including the percentage of INR results in the therapeutic range (PPINR) and the variability of the INR (63, 64). Multiple studies have reported that these parameters may provide additional prognostic information, apart from the TTR, regarding the risk of bleeding and thrombotic complications (65, 66). However, these findings have also been disputed by a recent study showing limited additional predictive value of INR variability when combined with TTR (67). Regardless of the used method to estimate therapeutic quality control, monitoring individual patients on VKA, based on the achieved quality of anticoagulation control during treatment, enables caretakers to adjust therapy accordingly.

Anticoagulant effects of statins

The interest for statins as potential anticoagulant treatment strategy was triggered in 2000 based on the results of the prospective cohort study from Grady and colleagues and further amplified by results from the Jupiter trial in 2009 (68,69). In this trial, rosuvastatin use was associated with a 43% reduction in the risk of a first VTE in healthy men and women with low cholesterol levels and high levels of C-reactive protein (67). Since this trial, multiple observational studies have also related statin use to decreased risk of both first and recurrent VTE (70-72). However, strong VTE risk reductions through statin use have also been disputed (73-75) Apart from epidemiological studies with clinical end points, animal studies and in vitro studies have also been performed linking statin use to anti-inflammatory and antithrombotic effects (76). However, there is currently no evidence from randomized studies that directly relate statin therapy to a reduced risk of recurrent VTE. An important reason for the lack of clinical trials in this area is that a clear pathophysiological mechanism through which statins could reduce VTE risk has not been established (77). Evidence from clinical trials and consequent meta-analyses relating statin use to changes in hemostatic thrombotic risk factors has been inconsistent, difficult to interpret due to the use of different statins in different dosages, likely subjected to publication bias, and not performed in the intended target population (e.g subjects at risk of recurrent VTE) (78-80).

We explored the anticoagulant effect of rosuvastatin in patients with a history of venous thrombosis after termination of anticoagulant treatment. The fact that statins do not increase the risk of bleeding makes them a potentially attractive therapeutic strategy for VTE prevention, especially in patients with a high risk of bleeding complications (82,83). In addition, these drugs may be considered as adjuvant therapy in patients who experienced recurrent VTE during anticoagulation therapy. Furthermore, insight in the anticoagulant effects of these drugs may also lead to new mechanistic insights, particularly in the crosstalk between inflammation, atherosclerosis and hemostasis (84,85).

In our START trial, we observed that rosuvastatin use did not affect arachidonic acid-mediated platelet reactivity (Chapter.11), but was associated with clear reductions in FVIII, FXI and VWF levels (Chapter 12) (86). High levels of these clotting factors have each been related to an increased VTE risk (87-90). Therefore, reductions in these clotting factors provide a pathophysiological rationale behind statin-mediated VTE risk reduction, if truly present. We observed no increases in other coagulation parameters associated with VTE risk, such as FVII or D-dimer levels. This suggests that rosuvastatin exerts a net anticoagulant effect, although we did not measure each of the individual

clotting factors. Similarly, maintenance dosages of both acenocoumarol and phenprocoumon also decreased after initiation of statin therapy in VKA users (Chapter 10), also suggesting a decrease in thrombin generation (91). A possibility to assess the net effect of rosuvastatin on the hemostatic balance would be to compare thrombin generation potential before and after initation of statin therapy. The net impact of these relatively small reductions in prothrombotic coagulation factors, in relation to recurrent VTE risk, is impossible predict. In order to definitively answer the question whether statins should be used for recurrent VTE prevention, an adequately powered randomized controlled trial with recurrent VTE as primary end point, should be conducted. Our studies support the conduction of such a trial from a pathophysiological perspective.

DOACs and future anticoagulant therapy strategies

Direct oral anticoagulants (DOACs) have been introduced over the last decade for several different thrombotic indications including atrial fibrillation and venous thrombosis. These agents reduce thrombin generation through either direct inhibition of thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, edoxaban). It is expected that these DOACs will increasingly replace VKAs for the majority of patients requiring anticoagulant therapy, due to their similar efficacy, combined with their comfort of use, predictable pharmacokinetics and their favorable bleeding risk profile compared to VKAs (92). These agents are associated with a significant reduction in the risk of intracranial bleeding, at the cost of a slight increased risk of gastro-intestinal bleeding (93). Due to the shift in bleeding localization, the risk of fatal bleeding is also lower during DOAC therapy compared to VKA therapy (93). The stable and predictable anticoagulant effect of DOACs ensures no routine requirement for monitoring of these agents (94,95). However, the lack of a need for monitoring may also be a potential pitfall. In case of suboptimal adherence or non-adherence, patients are exposed to an increased risk of thrombotic complications (96,97). Furthermore, since most patients requiring these drugs are older, decline in renal function and poor DOAC adherence due to decline in cognitive function, may also impose a threat to the continuous safe use of these drugs. Periodical) evaluation of patients treated with DOACs, including assessment of renal function, is warranted to ensure safe use of these agents. Although routine monitoring of DOAC concentrations is not required and currently not recommended, knowledge of DOAC concentrations in emergency settings may be useful. In case of major bleeding, urgent surgery or the need for thrombolysis in ischemic stroke patients, DOAC concentrations may directly influence the risk of bleeding and should therefore be incorporated in clinical decision making if possible (94). Measurement of DOAC plasma concentrations, however, is not always available,

let alone in emergency setting with a very limited time window. Point-of-care tests to measure DOAC concentrations or the anticoagulant effects of DOACs have been developed and may improve outcome of DOAC users in these emergency setting in the future (98, 99).

For patients with mechanical heart valves, dabigatran was shown to be inferior to warfarin, both in terms of efficacy (e.g. ischemic stroke risk) and safety (e.g. bleeding risk) (100). These patients need to be treated with VKA, since no other DOAC studies have been conducted in these patients. Therefore, future studies on optimal VKA management should specifically focus on patients with mechanical heart valves.

The superiority of DOACs over well-managed VKAs remains somewhat debatable since quality of anticoagulation control in several DOAC trials in patients with atrial fibrillation was suboptimal, directly affecting the risk of adverse events in the warfarin arms (101,102). Observational studies have shown a low risk of adverse events in well-managed warfarin patients, some even lower than reported in many of the DOAC trials (67,103,104). Furthermore, it has even been suggested that INR self-monitoring is a more cost-effective approach than treatment with dabigatran for long-term anticoagulant prophylaxis (105). Regardless of the superior efficacy of either VKA or DOACs, the risk of major and fatal bleeding complications during exposure, although lower with DOACs, remains present for both classes of drugs. Several developments are currently ongoing in the search for even safer anticoagulants with similar efficacy. The contact activation pathway, and especially factor XI, has been recognized as an attractive new target for anticoagulant drug development (106-108). In the landmark trial by Büller and colleagues, it was shown that a Factor XI antisense oligonucleotide was at least as effective as low-molecular-weight heparin in preventing venous thromboembolism in patients undergoing total knee arthroplasty, and, importantly, was associated with significantly fewer bleeding complications (109). Future clinical studies should demonstrate the efficacy and safety of FXI targeting drugs in the treatment of acute venous thrombosis or for stroke prophylaxis in patients with atrial fibrillation. Apart from factor XI, several other potential targets for antithrombotic treatment have been identified, predominantly agents affecting local and systemic inflammation (110). Increasing evidence suggests that inflammatory cytokines (interleukines) or a pro-inflammatory state, contributes to both the pathogenesis of atherothrombosis as well as venous thrombosis (85). Currently, different phase 3 and 4 trials are ongoing evaluating the effects of different anti-inflammatory agents (IL-1β IL-6, TNF-α blockers and low-dose methotrexate), on (recurrent) thrombotic events in patients with or at risk of cardiovascular disease (85). These studies and further insight

into the working mechanisms of these targets may eventually lead to the development of the ideal anticoagulant drug; a highly effective agent with (virtually) no bleeding complications. Until that time, the optimal use of current available agents is the best strategy for effective and safe anticoagulant treatment. Individual risk and benefit assessment should guide the choice for a particular anticoagulant strategy in close consultation with the patient.

REFERENCES

1. Biedermann JS, Leebeek FW, Buhre PN, de Lathouder S, Barends JP, de Maat MP, et al. Agreement between Coaguchek XS and STA-R Evolution (Hepato Quick) INR results depends on the level of INR. Thromb Res. 2015;136(3):652-7.
2. Plesch W, Wolf T, Breitenbeck N, Dikkeschei LD, Cervero A, Perez PL, et al. Results of the performance verification of the CoaguChek XS system. Thromb Res. 2008;123(2):381-9.
3. Plesch W, van den Besselaar AM. Validation of the international normalized ratio (INR) in a new point-of-care system designed for home monitoring of oral anticoagulation therapy. Int J Lab Hematol. 2009;31(1):20-5.
4. Wieloch M, Hillarp A, Strandberg K, Nilsson C, Svensson PJ. Comparison and evaluation of a Point-of-care device (CoaguChek XS) to Owren-type prothrombin time assay for monitoring of oral anticoagulant therapy with warfarin. Thromb Res. 2009;124(3):344-8.
5. Sobieraj-Teague M, Daniel D, Farrelly B, Coghlan D, Gallus A. Accuracy and clinical usefulness of the CoaguChek S and XS Point of Care devices when starting warfarin in a hospital outreach setting. Thromb Res. 2009;123(6):909-13.
6. Colella MP, Fiusa MM, Orsi FL, de Paula EV, Annichino-Bizzacchi JM. Performance of a point-of-care device in determining prothrombin time in an anticoagulation clinic. Blood Coagul Fibrinolysis. 2012;23(2):172-4.
7. Christensen TD, Larsen TB, Jensen C, Maegaard M, Sorensen B. International normalised ratio (INR) measured on the CoaguChek S and XS compared with the laboratory for determination of precision and accuracy. Thromb Haemost. 2009;101(3):563-9.
8. Lawrie AS, Hills J, Longair I, Green L, Gardiner C, Machin SJ, et al. The clinical significance of differences between point-of-care and laboratory INR methods in over-anticoagulated patients. Thromb Res. 2012;130(1):110-4.
9. Christensen TD, Larsen TB. Precision and accuracy of point-of-care testing coagulometers used for self-testing and self-management of oral anticoagulation therapy. J Thromb Haemost. 2012;10(2):251-60.
10. World Health Organisation. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy. WHO Expert Committee on Biological Standardization 1999:64-99.
11. Remijn JA, Lucas S, Wildeboer B, van Suijlen JD, Adriaansen HJ. Strongly increased international normalized ratio with recombinant Neoplastin R compared with tissue extract Neoplastin Plus in patients initiating oral anticoagulant therapy: implications for anticoagulation dosage. Clin Chem. 2008;54(11):1929-31.
12. Remijn JA, Wildeboer B, van Suijlen JD, Adriaansen HJ. Recombinant thromboplastins vs tissue-extract thromboplastins in patients on unstable oral anticoagulant therapy. Clin Chem. 2011;57(6):916-7.
13. van den Besselaar AM, Biedermann JS, Kruip MJ. Point-of-care testing and INR within-subject variation in patients receiving a constant dose of vitamin K antagonist. Thromb Haemost. 2015;114(5).
14. van Geest-Daalderop JH, Pequeriaux NC, van den Besselaar AM. Variability of INR in patients on stable long-term treatment with phenprocoumon and acenocoumarol and implications for analytical quality requirements. Thromb Haemost. 2009;102(3):588-92.
15. Fraser CG, Hyltoft Petersen P, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem. 1997;34 (Pt 1):8-12.
16. Fraser CG. Optimal analytical performance for point of care testing. Clin Chim Acta. 2001;307(1-2):37-43.
17. van den Besselaar AM, Fogar P, Pengo V, Palareti G, Braham S, Moia M, et al. Biological variation of INR in stable patients on long-term anticoagulation with warfarin. Thromb Res. 2012;130(3):535-7.

18. Biedermann JS, van Rein N, van den Besselaar AM, Buhre PN, de Maat MP, van der Meer FJ, et al. Impact of point-of-care international normalized ratio monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: a cohort study. J Thromb Haemost. 2016;14(4):695-703.
19. Shiach CR, Campbell B, Poller L, Keown M, Chauhan N. Reliability of point-of-care prothrombin time testing in a community clinic: a randomized crossover comparison with hospital laboratory testing. Br J Haematol. 2002;119(2):370-5.
20. Gadisseur AP, Breukink-Engbers WG, van der Meer FJ, van den Besselaar AM, Sturk A, Rosendaal FR. Comparison of the quality of oral anticoagulant therapy through patient self-management and management by specialized anticoagulation clinics in the Netherlands: a randomized clinical trial. Arch Intern Med. 2003;163(21):2639-46.
21. Gadisseur AP, Kaptein AA, Breukink-Engbers WG, van der Meer FJ, Rosendaal FR. Patient self-management of oral anticoagulant care vs. management by specialized anticoagulation clinics: positive effects on quality of life. J Thromb Haemost. 2004;2(4):584-91.
22. Health Quality O. Point-of-Care International Normalized Ratio (INR) Monitoring Devices for Patients on Long-term Oral Anticoagulation Therapy: An Evidence-Based Analysis. Ont Health Technol Assess Ser. 2009;9(12):1-114.
23. Heneghan CJ, Garcia-Alamino JM, Spencer EA, Ward AM, Perera R, Bankhead C, et al. Self-monitoring and self-management of oral anticoagulation. Cochrane Database Syst Rev. 2016;7:CD003839.
24. van den Besselaar AM, van der Meer FJ, Abdoel CF, Witteveen E. Analytical accuracy and precision of two novel Point-of-Care systems for INR determination. Thromb Res. 2015;135(3):526-31.
25. Kitchen DP, Kitchen S, Jennings I, Woods TA, Fitzmaurice DA, Murray ET, et al. Point of Care INR testing devices: performance of the Roche CoaguChek XS and XS Plus in the UK NEQAS BC external quality assessment programme for healthcare professionals: four years' experience. J Clin Pathol. 2012;65(12):1119-23.
26. Perry SL, Samsa GP, Ortel TL. Point-of-care testing of the international normalized ratio in patients with antiphospholipid antibodies. Thromb Haemost. 2005;94(6):1196-202.
27. Barcellona D, Fenu L, Vannini ML, Piras M, Marongiu F. Antiphospholipid syndrome patients: the performance of Coagucheck XS in the monitoring of Vitamin K-Antagonists. Thromb Res. 2012;129(4):e168-70.
28. Braham S, Novembrino C, Moia M, Torresani E, Tripodi A. Evaluation of a new PT-INR monitoring system in patients with the antiphospholipid syndrome. Int J Lab Hematol. 2016;38(5):497-504.
29. Della Valle P, Crippa L, Garlando AM, Pattarini E, Safa O, Vigano D'Angelo S, et al. Interference of lupus anticoagulants in prothrombin time assays: implications for selection of adequate methods to optimize the management of thrombosis in the antiphospholipid-antibody syndrome. Haematologica. 1999;84(12):1065-74.
30. Isert M, Miesbach W, Schuttfort G, Weil Y, Tirneci V, Kasper A, et al. Monitoring anticoagulant therapy with vitamin K antagonists in patients with antiphospholipid syndrome. Ann Hematol. 2015;94(8):1291-9.
31. Tripodi A, de Laat B, Wahl D, Ageno W, Cosmi B, Crowther M, et al. Monitoring patients with the lupus anticoagulant while treated with vitamin K antagonists: communication from the SSC of the ISTH. J Thromb Haemost. 2016;14(11):2304-7.
32. Federation of Dutch Thrombosis Services. Summary annual medical reports 2015. http://www.fnt.nl/ (Accessed April 2016).
33. van Geest-Daalderop JH, Hutten BA, Pequeriaux NC, Haas FJ, Levi M, Sturk A. The influence on INRs and coagulation factors of the time span between blood sample collection and intake of phenprocoumon or acenocoumarol: consequences for the assessment of the dose. Thromb Haemost. 2007;98(4):738-46.

34. Solvik UO, Roraas T, Petersen PH, Stavelin A, Monsen G, Sandberg S. The influence of coagulation factors on the in-treatment biological variation of international normalized ratio for patients on warfarin. Scand J Clin Lab Invest. 2014.
35. Smith SA, Comp PC, Morrissey JH. Phospholipid composition controls thromboplastin sensitivity to individual clotting factors. J Thromb Haemost. 2006;4(4):820-7.
36. Biedermann JS, van den Besselaar AM, de Maat MP, Leebeek FW, Kruip MJ. Monitoring of treatment with vitamin K antagonists: recombinant thromboplastins are more sensitive to factor VII than tissue-extract thromboplastins. J Thromb Haemost. 2017;15(3):500-6.
37. Gudmundsdottir BR, Francis CW, Bjornsdottir AM, Nellbring M, Onundarson PT. Critical role of factors II and X during coumarin anticoagulation and their combined measurement with a new Fiix-prothrombin time. Thromb Res. 2012;130(4):674-81.
38. Onundarson PT, Francis CW, Indridason OS, Arnar DO, Bjornsson ES, Magnusson MK, et al. Fiix-prothrombin time versus standard prothrombin time for monitoring of warfarin anticoagulation: a single centre, double-blind, randomised, non-inferiority trial. Lancet Haematol. 2015;2(6):e231-40.
39. Jonsson PI, Letertre L, Juliusson SJ, Gudmundsdottir BR, Francis CW, Onundarson PT. During warfarin induction, the Fiix-prothrombin time reflects the anticoagulation level better than the standard prothrombin time. J Thromb Haemost. 2017;15(1):131-9.
40. Barcellona D, Vannini ML, Fenu L, Balestrieri C, Marongiu F. Warfarin or acenocoumarol: which is better in the management of oral anticoagulants? Thromb Haemost. 1998;80(6):899-902.
41. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med. 1993;153(13):1557-62.
42. Gadisseur AP, van der Meer FJ, Adriaansen HJ, Fihn SD, Rosendaal FR. Therapeutic quality control of oral anticoagulant therapy comparing the short-acting acenocoumarol and the long-acting phenprocoumon. Br J Haematol. 2002;117(4):940-6.
43. Khan T, Wynne H, Wood P, Torrance A, Hankey C, Avery P, et al. Dietary vitamin K influences intra-individual variability in anticoagulant response to warfarin. Br J Haematol. 2004;124(3):348-54.
44. Rombouts EK, Rosendaal FR, Van Der Meer FJ. Daily vitamin K supplementation improves anticoagulant stability. J Thromb Haemost. 2007;5(10):2043-8.
45. Sconce E, Avery P, Wynne H, Kamali F. Vitamin K supplementation can improve stability of anticoagulation for patients with unexplained variability in response to warfarin. Blood. 2007;109(6):2419-23.
46. Gebuis EP, Rosendaal FR, van Meegen E, van der Meer FJ. Vitamin K1 supplementation to improve the stability of anticoagulation therapy with vitamin K antagonists: a dose-finding study. Haematologica. 2011;96(4):583-9.
47. Majeed H, Rodger M, Forgie M, Carrier M, Taljaard M, Scarvelis D, et al. Effect of 200muG/day of vitamin K1 on the variability of anticoagulation control in patients on warfarin: a randomized controlled trial. Thromb Res. 2013;132(3):329-35.
48. Lam J, Schulman S, Witt DM, Vandvik PO, Qayyum F, Holbrook AM. Anticoagulation control with daily low-dose vitamin k to reduce clinically adverse outcomes and international normalized ratio variability: a systematic review and meta-analysis. Pharmacotherapy. 2013;33(11):1184-90.
49. Mahtani KR, Heneghan CJ, Nunan D, Roberts NW. Vitamin K for improved anticoagulation control in patients receiving warfarin. Cochrane Database Syst Rev. 2014(5):CD009917.
50. Holbrook A, Schulman S, Witt DM, Vandvik PO, Fish J, Kovacs MJ, et al. Evidence-based management of anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e152S-84S.
51. Whitlock RP, Fordyce CB, Midei MG, Ellis D, Garcia D, Weitz JI, et al. A randomised, double blind comparison of tecarfarin, a novel vitamin K antagonist, with warfarin. The EmbraceAC Trial. Thromb Haemost. 2016;116(2):241-50.

52. Leendertse AJ, Egberts AC, Stoker LJ, van den Bemt PM, Group HS. Frequency of and risk factors for preventable medication-related hospital admissions in the Netherlands. Arch Intern Med. 2008;168(17):1890-6.
53. Douketis JD, Spyropoulos AC, Spencer FA, Mayr M, Jaffer AK, Eckman MH, et al. Perioperative management of antithrombotic therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e326S-50S.
54. Spyropoulos AC, Al-Badri A, Sherwood MW, Douketis JD. Periprocedural management of patients receiving a vitamin K antagonist or a direct oral anticoagulant requiring an elective procedure or surgery. J Thromb Haemost. 2016;14(5):875-85.
55. Dutch Society for Internal Medicine: Guideline antithrombotic therapy. http://richtlijnendatabase.nl/richtlijn/antitrombotisch_beleid/perioperatief_beleid_bij_antistolling.html. Accessed August 15, 2016.
56. Dreijer AR, Kruip MJ, Diepstraten J, Polinder S, Brouwer R, Leebeek FW, et al. Antithrombotic stewardship: a multidisciplinary team approach towards improving antithrombotic therapy outcomes during and after hospitalisation: a study protocol. BMJ Open. 2016;6(12):e011537.
57. van Rein N, Lijfering WM, Bos MH, Herruer MH, Vermaas HW, van der Meer FJ, et al. Objectives and Design of BLEEDS: A Cohort Study to Identify New Risk Factors and Predictors for Major Bleeding during Treatment with Vitamin K Antagonists. PLoS One. 2016;11(12):e0164485.
58. Trujillo-Santos J, Bergmann JF, Bortoluzzi C, Lopez-Reyes R, Giorgi-Pierfranceschi M, Lopez-Saez JB, et al. Once versus twice daily enoxaparin for the initial treatment of acute venous thromboembolism. J Thromb Haemost. 2017;15(3):429-38.
59. van Dongen CJ, MacGillavry MR, Prins MH. Once versus twice daily LMWH for the initial treatment of venous thromboembolism. Cochrane Database Syst Rev. 2005:CD003074.
60. Bhutia S, Wong PF. Once versus twice daily low molecular weight heparin for the initial treatment of venous thromboembolism. Cochrane Database Syst Rev. 2013:CD003074
61. van den Besselaar AM, Biedermann JS, van der Meer FJ, Adriaansen HJ, Leebeek FW, Kruip MJ. Control of anticoagulation with vitamin K antagonists: overestimation of median time in therapeutic range when assessed by linear interpolation. Thromb Haemost. 2016;116(4):679-86.
62. van den Besselaar AM. Recommended method for reporting therapeutic control of oral anticoagulant therapy. Control of Anticoagulation Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost. 1990;63(2):316-7.
63. Lind M, Fahlen M, Kosiborod M, Eliasson B, Oden A. Variability of INR and its relationship with mortality, stroke, bleeding and hospitalisations in patients with atrial fibrillation. Thromb Res. 2012;129(1):32-5.
64. Chan PH, Li WH, Hai JJ, Chan EW, Wong IC, Tse HF, et al. Time in Therapeutic Range and Percentage of International Normalized Ratio in the Therapeutic Range as a Measure of Quality of Anticoagulation Control in Patients With Atrial Fibrillation. Can J Cardiol. 2016;32(10):1247.e23-.e28.
65. Razouki Z, Ozonoff A, Zhao S, Jasuja GK, Rose AJ. Improving quality measurement for anticoagulation: adding international normalized ratio variability to percent time in therapeutic range. Circ Cardiovasc Qual Outcomes. 2014;7(5):664-9.
66. Razouki Z, Burgess JF, Jr., Ozonoff A, Zhao S, Berlowitz D, Rose AJ. Improving Anticoagulation Measurement Novel Warfarin Composite Measure. Circ Cardiovasc Qual Outcomes. 2015;8(6):600-7.
67. Bjorck F, Renlund H, Lip GY, Wester P, Svensson PJ, Sjalander A. Outcomes in a Warfarin-Treated Population With Atrial Fibrillation. JAMA Cardiol. 2016;1(2):172-80.
68. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, et al. Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The Heart and Estrogen/progestin Replacement Study. Ann Intern Med. 2000;132(9):689-96.

69. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360(18):1851-61.
70. Schmidt M, Cannegieter SC, Johannesdottir SA, Dekkers OM, Horvath-Puho E, Sorensen HT. Statin use and venous thromboembolism recurrence: a combined nationwide cohort and nested case-control study. J Thromb Haemost. 2014;12(8):1207-15.
71. Ashrani AA, Barsoum MK, Crusan DJ, Petterson TM, Bailey KR, Heit JA. Is lipid lowering therapy an independent risk factor for venous thromboembolism? A population-based case-control study. Thromb Res. 2015;135(6):1110-6.
72. Smith NL, Harrington LB, Blondon M, Wiggins KL, Floyd JS, Sitlani CM, et al. The association of statin therapy with the risk of recurrent venous thrombosis. J Thromb Haemost. 2016;14(7):1384-92.
73. Rahimi K, Bhala N, Kamphuisen P, Emberson J, Biere-Rafi S, Krane V, et al. Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. PLoS Med. 2012;9(9):e1001310.
74. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? PLoS Med. 2012;9(9):e1001311.
75. Cushman M. A new indication for statins to prevent venous thromboembolism? Not yet. J Thromb Haemost. 2009;7(4):511-3.
76. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. Circulation. 2013;127(2):251-7.
77. Lijfering WM, Biedermann JS, Kruip MJ, Leebeek FW, Rosendaal FR, Cannegieter SC. Can we prevent venous thrombosis with statins: an epidemiologic review into mechanism and clinical utility. Expert review of hematology. 2016;9(11):1023-30.
78. Sahebkar A, Serban C, Mikhailidis DP, Undas A, Lip GY, Muntner P, et al. Association between statin use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials. Thromb Haemost. 2015;114(3):546-57.
79. Sahebkar A, Serban C, Ursoniu S, Mikhailidis DP, Undas A, Lip GY, et al. The impact of statin therapy on plasma levels of von Willebrand factor antigen. Systematic review and meta-analysis of randomised placebo-controlled trials. Thromb Haemost. 2016;115(3):520-32.
80. Sahebkar A, Catena C, Ray KK, Vallejo-Vaz AJ, Reiner Z, Sechi LA, et al. Impact of statin therapy on plasma levels of plasminogen activator inhibitor-1. A systematic review and meta-analysis of randomised controlled trials. Thromb Haemost. 2016;116(1):162-71.
81. Bianconi V, Sahebkar A, Banach M, Pirro M. Statins, haemostatic factors and thrombotic risk. Current opinion in cardiology. 2017.
82. Wells PS, Gebel M, Prins MH, Davidson BL, Lensing AW. Influence of statin use on the incidence of recurrent venous thromboembolism and major bleeding in patients receiving rivaroxaban or standard anticoagulant therapy. Thromb J. 2014;12:26.
83. Badillo R, Schmidt R, Mortensen EM, Frei CR, Mansi I. Statin therapy and gastrointestinal hemorrhage: a retrospective cohort study with propensity score-matching. Pharmacoepidemiol Drug Saf. 2015;24(8):849-57.
84. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, et al. An association between atherosclerosis and venous thrombosis. N Engl J Med. 2003;348(15):1435-41.
85. De Caterina R, D'Ugo E, Libby P. Inflammation and thrombosis - testing the hypothesis with anti-inflammatory drug trials. Thromb Haemost. 2016;116(6):1012-21.
86. Biedermann JS, Cannegieter SC, Roest M, van der Meer FJ, Reitsma PH, Kruip MJ, et al. Platelet reactivity in patients with venous thrombosis who use rosuvastatin: a randomized controlled clinical trial. J Thromb Haemost. 2016;14(7):1404-9.
87. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet. 1995;345(8943):152-5.
88. Kyrle PA, Minar E, Hirschl M, Bialonczyk C, Stain M, Schneider B, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med. 2000;343(7):457-62.

89. Kamphuisen PW, Eikenboom JC, Rosendaal FR, Koster T, Blann AD, Vos HL, et al. High factor VIII antigen levels increase the risk of venous thrombosis but are not associated with polymorphisms in the von Willebrand factor and factor VIII gene. Br J Haematol. 2001;115(1):156-8.
90. Nossent AY, van Marion V, van Tilburg NH, Rosendaal FR, Bertina RM, van Mourik JA, et al. von Willebrand factor and its propeptide: the influence of secretion and clearance on protein levels and the risk of venous thrombosis. J Thromb Haemost. 2006;4(12):2556-62.
91. van Rein N, Biedermann JS, Bonafacio SM, Kruip MJ, van der Meer FJ, Lijfering WM. Statin use decreases coagulation in users of vitamin K antagonists. European journal of clinical pharmacology. 2016;72(12):1441-7.
92. Sterne JA, Bodalia PN, Bryden PA, Davies PA, Lopez-Lopez JA, Okoli GN, et al. Oral anticoagulants for primary prevention, treatment and secondary prevention of venous thromboembolic disease, and for prevention of stroke in atrial fibrillation: systematic review, network meta-analysis and cost-effectiveness analysis. Health Technol Assess. 2017;21(9):1-386.
93. Skaistis J, Tagami T. Risk of Fatal Bleeding in Episodes of Major Bleeding with New Oral Anticoagulants and Vitamin K Antagonists: A Systematic Review and Meta-Analysis. PLoS One. 2015;10(9):e0137444.
94. Drouet L, Bal Dit Sollier C, Steiner T, Purrucker J. Measuring non-vitamin K antagonist oral anticoagulant levels: When is it appropriate and which methods should be used? International journal of stroke : official journal of the International Stroke Society. 2016;11(7):748-58.
95. Salmonson T, Dogne JM, Janssen H, Garcia Burgos J, Blake P. Non-vitamin-K oral anticoagulants and laboratory testing: now and in the future: Views from a workshop at the European Medicines Agency (EMA). European heart journal Cardiovascular pharmacotherapy. 2017;3(1):42-7.
96. Shore S, Carey EP, Turakhia MP, Jackevicius CA, Cunningham F, Pilote L, et al. Adherence to dabigatran therapy and longitudinal patient outcomes: insights from the veterans health administration. American heart journal. 2014;167(6):810-7.
97. Yao X, Abraham NS, Alexander GC, Crown W, Montori VM, Sangaralingham LR, et al. Effect of Adherence to Oral Anticoagulants on Risk of Stroke and Major Bleeding Among Patients With Atrial Fibrillation. J Am Heart Assoc. 2016;5(2).
98. Harenberg J, Du S, Wehling M, Zolfaghari S, Weiss C, Kramer R, et al. Measurement of dabigatran, rivaroxaban and apixaban in samples of plasma, serum and urine, under real life conditions. An international study. Clinical chemistry and laboratory medicine. 2016;54(2):275-83.
99. Bliden KP, Chaudhary R, Mohammed N, Muresan AA, Lopez-Espina CG, Cohen E, et al. Determination of non-Vitamin K oral anticoagulant (NOAC) effects using a new-generation thrombelastography TEG 6s system. J Thromb Thrombolysis. 2017.
100. Eikelboom JW, Connolly SJ, Brueckmann M, Granger CB, Kappetein AP, Mack MJ, et al. Dabigatran versus warfarin in patients with mechanical heart valves. N Engl J Med. 2013;369(13):1206-14.
101. Wallentin L, Yusuf S, Ezekowitz MD, Alings M, Flather M, Franzosi MG, et al. Efficacy and safety of dabigatran compared with warfarin at different levels of international normalised ratio control for stroke prevention in atrial fibrillation: an analysis of the RE-LY trial. Lancet. 2010;376(9745):975-83.
102. Ansell J. New oral anticoagulants should not be used as first-line agents to prevent thromboembolism in patients with atrial fibrillation. Circulation. 2012;125(1):165-70; discussion 70.
103. Wieloch M, Sjalander A, Frykman V, Rosenqvist M, Eriksson N, Svensson PJ. Anticoagulation control in Sweden: reports of time in therapeutic range, major bleeding, and thrombo-embolic complications from the national quality registry AuriculA. European heart journal. 2011;32(18):2282-9.

104. Sjogren V, Grzymala-Lubanski B, Renlund H, Friberg L, Lip GY, Svensson PJ, et al. Safety and efficacy of well managed warfarin. A report from the Swedish quality register Auricula. Thromb Haemost. 2015;113(6):1370-7.
105. Carles M, Brosa M, Souto JC, Garcia-Alamino JM, Guyatt G, Alonso-Coello P. Cost-effectiveness analysis of dabigatran and anticoagulation monitoring strategies of vitamin K antagonist. BMC health services research. 2015;15:289.
106. Gailani D, Bane CE, Gruber A. Factor XI and contact activation as targets for antithrombotic therapy. J Thromb Haemost. 2015;13(8):1383-95.
107. Gailani D, Gruber A. Factor XI as a Therapeutic Target. Arterioscler Thromb Vasc Biol. 2016;36(7):1316-22.
108. Wheeler AP, Gailani D. The Intrinsic Pathway of Coagulation as a Target for Antithrombotic Therapy. Hematol Oncol Clin North Am. 2016;30(5):1099-114.
109. Buller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, et al. Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med. 2015;372(3):232-40.
110. Ridker PM, Luscher TF. Anti-inflammatory therapies for cardiovascular disease. European heart journal. 2014;35(27):1782-91.

CHAPTER 14

Summary

SUMMARY

Patients at risk or suffering from arterial or venous thrombosis require anticoagulant therapy to prevent the onset, progression or recurrence of thrombotic events. Although currently available agents are effective, their protective effects come at a high cost, since patients are exposed to an increased bleeding risk during treatment. We have performed several studies focusing on current monitoring and management of anticoagulation therapy, in order to improve the safety and efficacy of monitoring and management of anticoagulant agents in the future.

The first part of this this focuses on the monitoring of treatment with vitamin K antagonists (VKA). In **chapter 1**, the general introduction, we present an overview of current monitoring and management of anticoagulation therapy. Next, in **chapter 2,** we compared the analytical and clinical agreement between point-of-care (CoaguChek XS) and laboratory (STA-R Evolution, Hepato Quick) INR results in a cohort of over 3000 patients treated with VKA. We found that INR results from both methods correlated strongly (R=0.901, P<0.001), especially in the therapeutic INR range. The numerical difference between methods increased with increasing INR. The mean INR difference between methods ranged from -0.18 (95%CI, -0.20 to -0.16) INR point for point-of-care results 2.0-3.0, up to 1.14 (95%CI, 0.87 to 1.42) INR point for point-of-care results 7.1-8.0. Overall, clinical agreement regarding therapeutic range was generally satisfactory (88%), but disagreement did occur at both subtherapeutic and supratherapeutic INR levels. In **chapter 3**, we assessed the within-subject biological INR variation (CV in %) in self-monitoring (n=322) and non-self-monitoring (n=123) patients who received a stable dose of either acenocoumarol or phenprocoumon. All patients monitored themselves or were monitored by staff using the CoaguCheck XS system. In the self-monitoring patients, median within-subject CV was significantly higher in patients on acenocoumarol than in patients on phenprocoumon (10.2 vs. 8.6%, P=0.001). In patients receiving low-intensity acenocoumarol no difference in median within-subject CV was observed between self-monitoring and non-self-monitoring patients (10.4 vs. 10.2%, P=0.690). The approximate imprecision coefficient of variation of the CoaguChek XS system as reported in literature (4%), should not exceed half of the observed biological variation in any of the studied patient groups in order to be suitable for monitoring these patients. Based on the biological INR variation we observed in this study, the analytical performance of the CoaguChek XS system is satisfactory for INR monitoring in these patients. Next, **in chapter 4**, we investigated the impact of point-of-care (POC) INR monitoring on quality of treatment in non-self-monitoring patients on VKA. We evaluated both the quality of anticoagulation control, as well as clinical outcome, by

comparing two non-self-monitoring patient cohorts of almost 2000 patients. Regarding the quality of anticoagulation control, median time in therapeutic range (TTR%) was significantly lower during POC monitoring (77.9% [95%CI, 67.2 to 87.4]) than during laboratory monitoring (81.0%, [95%CI, 71.1 to 90.5]; P<0.001). Similarly, the percentage of patients with poor anticoagulation control (TTR<60%) was higher during POC monitoring than during laboratory monitoring (10.7% vs. 14.5%, P<0.001). Overall, median TTR remained satisfactory according to Dutch guideline recommendations. We found no differences in the risk of major bleeding, ischemic stroke, hospitalization and all-cause mortality between the two cohorts (all adjusted hazard rates around unity), indicating that point-of-care INR monitoring was safe in these patients and non-inferior to standard laboratory monitoring regarding clinical outcome. In **chapter 5** we explored the sensitivity of 6 different commercial thromboplastin reagents (three human recombinant and three tissue-derived) to Factor VII (FVII). We spiked five different pooled plasma samples of patients on VKA with three different concentration of purified human FVII (0.006, 0.012, and 0.062 µg/mL plasma) or buffer as control. We measured dose-dependent INR decreases after spiking with FVII with all six reagents. A significant difference in FVII-induced proportional INR change was observed between the six thromboplastins (P=0.004). In contrast, proportional INR changes did not differ amongst the tissue-derived (P=0.575) or the recombinant (P=0.085) reagents. Pooling of the data from the two thromboplastin types (e.g. human recombinant vs. tissue-derived) revealed that FVII-induced INR changes were significantly greater when measured with a recombinant reagent. These data confirm that recombinant human thromboplastins are more sensitive to FVII than tissue-derived thromboplastins.

The second part of this thesis focuses on the management of anticoagulation therapy. In **chapter 6** we evaluated the major bleeding risk of different low-molecular-weight heparins (LMWH) during combined treatment with VKA in almost 13,000 out-hospital patients with new onset venous thrombosis. Overall, the risk of major bleeding was low with a cumulative incidence of 2.5 per 1000 patients (95%CI, 1.7 to 3.5). Cumulative incidences of major bleeding did not significantly differ between LMWHs using nadroparin once daily as reference, and ranged from 2.1 per 1000 patients for nadroparin once daily to 3.8 per 1000 patients for enoxaparin. A trend towards an increased major bleeding risk was observed when comparing once daily with twice-daily nadroparin dosing (relative risk 1.98; 95%CI, 0.76 to 5.14). The absolute increased risk of major bleeding associated with twice daily nadroparin dosing was 0.13% resulting in a number needed to harm of 767. Next, in **chapter 7**, we studied the agreement between two different methods for assessment of therapeutic quality control in different patient groups on VKA. The percentage of time in therapeutic range (TTR%)

was compared with the cross-sectional proportion of INR results within the therapeutic range (CSP%) using data reported by members of the Federation of Dutch Thrombosis Services between 2010 and 2013. Overall, TTR results were slightly, but consistently higher than CSP results. Good correlation was observed between methods in patients on acenocoumarol and excellent correlation between methods in patients on phenprocoumon. In long-term patients on acenocoumarol, median TTR was significantly higher than CSP (80.0% vs. 78.7% respectively, P<0.001), while patients on phenprocoumon no difference between methods was observed. The differences between methods in patients on acenocoumarol in contrast to patients on phenprocoumon may be explained by differences in INR variability between consecutive INR results within the therapeutic range. In **chapter 8,** we evaluated the perioperative management strategy, identified predictors of post-procedural oral cavity bleeding and assessed clinical outcome after 2329 dental procedures performed in over 2000 patients VKA. We found that guideline adherence was suboptimal as demonstrated by heterogeneous VKA management. Patients undergoing low-risk dental procedures during which VKA was continued in combination with a local prohemostatic agents (tranexamic acid mouthwash) in line with local guidelines, had a significantly lower bleeding risk to those where VKA was continued without a prohemostatic agent (OR=0.41; 95%CI, 0.23 to 0.73) or where VKA was interrupted and bridged with LMWH (OR=0.49; 95%CI, 0.24 to 1.00) and not a significant increased risk compared to complete interruption of anticoagulant therapy (OR 1.44; 95%CI, 0.62 to 3.64). In contrast, VKA continuation was associated with a 3-fold increased bleeding risk after high-risk dental procedures compared to VKA interruption. Multivariate analysis revealed that most risk factors for post-procedural bleeding were specifically related to the followed perioperative management strategy. The risk of thromboembolic complications after dental procedures was low (0.2%) and not related to management strategy or procedure risk.

The third and final part of this thesis focuses on the anticoagulant effects of statins and their potential use as an alternative and safer antithrombotic treatment strategy for patients with venous thromboembolism. First, in **chapter 9**, currently available evidence for prevention of (recurrent) venous thromboembolism with statins is reviewed from an epidemiological perspective, specifically addressing potential forms of bias in prior clinical studies. Next in **chapter 10**, we performed a retrospective cohort study, evaluating the impact of initiation of statin therapy on VKA maintenance dosage in 435 patients on phenprocoumon and 303 patients on acenocoumarol. Maintenance dosages before initiation of statin treatment were compared with maintenance dosages immediately, 6 weeks and 12 weeks after initiation of statin treatment. In

phenprocoumon users, mean dosages were 0.02 (95% CI, 0.00 to 0.03), 0.03 (95% CI, 0.01 to 0.05) and 0.07 mg/day (95% CI, 0.04 to 0.09) lower compared to the mean dosage before treatment. In acenocoumarol users mean dosages were 0.04 (95%CI, 0.01 to 0.07), 0.10 (95%CI, 0.03 to 0.16) and 0.11 (95% CI, 0.04 to 0.18) mg/day lower respectively. Statin therapy was therefore associated with minor, but statistically significant, changes in immediate and long-term VKA maintenance dosages, irrespective of VKA type. Although these dosage changes were of little clinical relevance by themselves, these might be explained by anticoagulant effects op statins. In **chapters 11 and 12** we performed a randomized controlled clinical trial evaluating the short-term (1 month) effect of rosuvastatin therapy on platelet reactivity and several markers of coagulation in patients with a history of deep vein thrombosis or pulmonary embolism. In the 'statins reduce thrombophilia' (START) trial, patients with an objectified venous thrombotic event, who were allowed to discontinue VKA treatment after 3-12 months, were included. First in **chapter 11**, we measured platelet reactivity, before and after rosuvastatin therapy or no intervention, in 50 consecutive patients included in the START trial. Arachidonic acid mediated platelet reactivity, in platelet reaction units (PRU), was measured using the VerifyNow system. In total, 47/50 patients (94.0%) had valid baseline and end of study PRU measurements. We observed no difference in mean PRU at end of study (mean PRU 613) compared to baseline (mean PRU 609) in rosuvastatin users (mean change 5; 95% CI, -18 to 27). Similar results were observed in non-users. Furthermore, there was no difference in PRU change between rosuvastatin users and non-users (mean difference in change 6, 95% CI, -20 to 33). After exclusion of patients using antiplatelet drugs or with thrombocytopenia, similar results were obtained (mean difference in change -1; 95% CI, -20 to 19). Therefore, rosuvastatin therapy did not affect arachidonic acid mediated platelet reactivity in patients with a history of venous thrombosis. In **chapter 12**, we investigated the effect of rosuvastatin therapy on coagulation parameters associated with liver function (clotting factors VII and XI), fibrinolysis (D-dimer) and endothelial function (Factor VIII and Von Willebrand factor). In total, 255 patients, of whom 131 to rosuvastatin and 124 to no intervention, were included between December 2012 and December 2016. Eight participants (5 rosuvastatin users and 3 non-users) did not complete follow-up. In total 126 rosuvastatin users and 121 non-users were included for final analysis. At baseline, no differences in coagulation parameters were observed between rosuvastatin users and non-users. At end of study, mean factor VIII levels were significantly decreased in rosuvastatin users (-7.2 IU/dL, (95% CI -11.5 to -2.9). No change in Factor VIII was observed in non-users (mean difference -0.1; 95% CI -3.0 to 2.9). Adjustment for age and sex did not change these findings. Similarly factor XI levels also decreased in rosuvastatin users (-5.9 IU/dL, (95% CI -9.0 to -2.7). Log-transformed D-dimer levels were higher at end of

study in non-users (mean difference 0.15, 95% CI 0.02 to 0.29), but unchanged in rosuvastin users (mean difference 0.01, 95% CI -0.08 to 0.10). No significant changes in Factor VII and Von Willebrand Factor were seen in the intention-to-treat analysis. Restriction analysis revealed that, after exclusion of participants reporting an infection during the study, Von Willebrand Factor antigen levels also decreased in rosuvastatin users (mean change -7.0; 95% CI -12.7 to -3.2), but not in non-users (mean change -1.0; 95% CI -4.3 to 2.4). Prespecified subgroup analyses revealed similar results as the main analyses. However, the anticoagulant effects of rosuvastatin were more pronounced in participants with deep vein thrombosis, unprovoked venous thrombosis or with cardiovascular risk factors compared to participants with pulmonary embolism, provoked venous thrombosis or in participants without cardiovascular risk factors. In conclusion, rosuvastatin use was associated with reductions in several coagulation factors that are associated with an increased risk of venous thrombosis. These anticoagulant effects of rosuvastatin provide a rationale for statin-mediated risk reduction of venous thrombosis. An adequately powered randomized controlled trial should be conducted to definitively answer whether rosuvastatin should be used for the prevention of recurrent venous thrombosis. Finally, in **chapter 13** we discussed the findings of the studies described in this thesis and give suggestions for further studies to optimize anticoagulant therapy in the future.

NEDERLANDSE SAMENVATTING

Patiënten die risico lopen op of lijden aan arteriële of veneuze trombose behoeven behandeling met antistolling om het ontstaan, de progressie of het terugkeren van trombose te voorkomen. Hoewel de bestaande middelen hiervoor effectief zijn, hebben zij het nadeel dat patiënten een verhoogd bloedingsrisico hebben tijdens de behandeling. Wij hebben verschillende studies uitgevoerd die zich richtten op de huidige monitoring en het management van antistollingsbehandeling. Hiermee kan mogelijk de veiligheid en effectiviteit van antistollingsbehandeling in de toekomst verbeterd worden.

In **hoofdstuk 1**, de algemene introductie, presenteren wij een overzicht van de huidige manier van monitoren en managen van antistolling therapie.

Vervolgens hebben wij in **hoofdstuk 2** de analytische en klinische overeenkomstigheid van INR-resultaten, verkregen middels een point-of-care apparaat (CoaguChek XS) en een laboratoriumbepaling (STA-R Evolution, Hepato Quick), vergeleken in een cohort van ruim 3000 patiënten op vitamine K antagonisten. Wij vonden een sterke correlatie tussen de uitslagen van deze 2 methoden (R=0.901, P<0.001), met name in het therapeutische INR-gebied. De numerieke verschillen tussen de methoden namen toe naarmate de INR hoger werd. Het gemiddelde verschil tussen de methoden liep van -0.18 INR punt (95% BI, -0.20 tot -0.16) voor resultaten met een point-of-care INR tussen de 2.0 en 3.0, tot 1.14 INR punt (95% BI, 0.87 tot 1.42) bij point-of-care resultaten tussen de 7.1 en 8.0. De klinische overeenkomstigheid tussen de resultaten met betrekking tot of de uitslag, in, onder of boven het therapeutische gebied van de patiënt lag, was adequaat (88%), hoewel het relatief frequent voorkwam, zowel bij te lage als te hoge uitslagen, dat er klinisch geen overeenkomstigheid was tussen INR-resultaten.

In **hoofdstuk 3** hebben we de biologische binnen-persoons variatie (CV%) in zelfmetende (n=322) en niet-zelfmetende (n=123) patiënten, die een stabiele dosis acenocoumarol of fenprocoumon kregen geanalyseerd. Al deze patiënten werden gemonitord of monitorden zichzelf met het CoaguChek XS systeem. In zelfmetende patiënten was de mediane binnen-persoons variatie significant hoger in patiënten op acenocoumarol dan in patiënten op fenprocoumon (10.2 vs. 8.6%, P=0.001). Bij patiënten op acenocoumarol was geen verschil in mediane binnen-persoons variatie tussen zelfmetende en niet zelfmetende patiënten (10.4 vs. 10.2%, P=0.690). De variatiecoëfficiënt van de CoaguChek XS (4%), zoals gerapporteerd in de literatuur, zou niet hoger moeten zijn dan de helft van de biologische variatie om geschikt te zijn voor

gebruik in de bestudeerde patiëntgroepen. Op basis van de biologische INR-variatie die wij vonden in onze studie, voldoet de CoaguChek aan dit criterium en is deze methode precies genoeg voor de monitoring van deze patiënten.

Vervolgens hebben wij in **hoofdstuk 4** het effect van de invoering van routine point-of-care (POC) INR-bepaling op de kwaliteit van zorg in niet-zelfmetende patiënten op vitamine K-antagonisten bestudeerd. Hierbij hebben wij zowel naar de kwaliteit van instelling van de patiënten gekeken als naar het risico op complicaties. Dit hebben wij gedaan door twee cohorten van ongeveer 2000 niet-zelfmetende patiënten met elkaar te vergelijken. Met betrekking tot de therapeutische instelling zagen wij dat het mediane percentage tijd in het therapeutische gebied (TTR%) significant lager was gedurende POC INR-bepaling (77.9% [95% BI, 67.2 tot 87.4]) dan tijdens laboratorium INR-bepaling (81.0%, [95% BI, 71.1 tot 90.5]; P<0.001). Ook het percentage patiënten dat een slechte therapeutische instelling had (TTR<60%) was hoger tijdens POC INR bepaling dan tijdens laboratorium INR-bepaling (10.7% vs. 14.5%, P<0.001). De mediane TTR tijdens POC INR-bepaling bleef echter adequaat en conform aanbevelingen in de Nederlandse richtlijnen. Wij vonden geen verschil in het risico op een majeure bloeding, herseninfarct, ziekenhuisopname of overlijden tussen de twee cohorten. Dit suggereert dat POC INR-controle net zo veilig is als laboratorium INR-controle met betrekking tot het risico op complicaties.

In **hoofdstuk 5** hebben wij van 6 verschillende commerciële thromboplastines (waarvan 3 humaan recombinant en 3 weefselextract) de gevoeligheid voor Factor VII (FVII) vergeleken. Wij hebben 3 verschillende hoeveelheden humaan FVII (0.006, 0.012, en 0.062µg/mL plasma) en buffer als negatieve controle, aan 5 gepoolde patiënten plasma's toegevoegd. Wij zagen dosis-afhankelijke INR-verlagingen bij alle 6 gebruikte thromboplastines. Er was een significant verschil in de FVII-geïnduceerde INR- verlaging tussen de 6 thromboplastines (P=0.004). Daarentegen zagen wij geen verschillen tussen de recombinante thromboplastines onderling (P=0.575) of de weefselextract thromboplastines onderling (P=0.085). Samenvoeging van de data van de twee type thromboplastines (recombinant en weefselextract) toonde aan dat de FVII-geïnduceerde INR-verlaging sterker was wanneer een recombinant humaan thromboplastine werd gebruikt dan wanneer een weefsel extract thromboplastine werd gebruikt. Deze data bevestigt dat recombinant humane thrombomplastines gevoeliger zijn voor FVII dan weefselextract thromboplastines.

In **hoofdstuk 6** hebben we gekeken of er verschillen zijn in het risico op een majeure bloeding tussen verschillende laag-moleculair-gewichts heparines (LMWH) bij gelijktijdig

gebruik met een vitamine K-antagonist. Wij hebben dit onderzocht in een cohort van bijna 13.000 ambulante patiënten die recent een veneuze trombose hadden gehad. Het risico op een ernstige bloeding was laag met een cumulatieve incidentie van 2.5 per 1000 patiënten (95% BI, 1.7 tot 3.5). Er was geen onderling verschil in cumulatieve incidentie tussen de LMWH's wanneer men nadroparine eenmaal daags als referentie gebruikte. De cumulatieve incidentie liep van 2.1 per 1000 patiënten voor eenmaal daags nadroparine tot 3.8 bij het gebruik van enoxaparine. Het bloedingsrisico leek wel hoger bij tweemaal daags gebruik van LMWH vergeleken met eenmaal daags gebruik (relatief risico van 1.98, 95% BI 0.76 tot 5.14). De absolute toename in bloedingsrisico bij tweemaal daags nadroparine gebruik was 0.13%, met een number needed to harm van 767.

Vervolgens hebben wij in **hoofdstuk 7** gekeken naar de overeenkomstigheid tussen 2 verschillende methoden die gebruikt worden voor het meten van de therapeutische instelling van patiënten op VKA. Wij hebben dit in verschillende patiëntengroepen gedaan. Het percentage tijd in de therapeutische range (TTR%) werd hierbij vergeleken met de proportie patiënten van wie de laatste INR in het therapeutisch gebied was (CSP %). Hiervoor werd gebruik gemaakt van data gerapporteerd tussen 2010 en 2013 door leden van de Federatie van Nederlandse trombosediensten. Over het algemeen waren de TTR resultaten gemiddeld consequent iets hoger dan de CSP resultaten. In long-term patiënten op acenocoumarol was de mediane TTR significant hoger dan de CSP (80.0% vs. 78.7%, P<0.001). Bij long-term patiënten op fenprocoumon was er daarentegen geen verschil tussen beide methoden. De verschillen tussen de methoden in patiënten op acenocoumarol kunnen mogelijk verklaard worden door verschillen in de variabiliteit van de INR tussen opeenvolgende metingen binnen de therapeutische range.

In **hoofdstuk 8** hebben wij het perioperatieve antistollingsbeleid, risicofactoren voor nabloedingen en de klinische uitkomsten na 2329 tandheelkundige ingrepen in ruim 2000 patiënten die behandeld werden met vitamine K-antagonisten geëvalueerd. De richtlijnen rondom deze ingrepen werden vaak niet opgevolgd, hetgeen zich uitte in heterogeen perioperatief antistollingsbeleid. Patiënten die een laag-risico tandheelkundige ingreep ondergingen, waarbij conform de richtlijn de VKA gecontinueerd werd in combinatie met tranexaminezuur mondspoeling, hadden een lager bloedingsrisico vergeleken met patiënten waarbij geen tranexaminezuur was gegeven (OR) of patiënten waarbij de VKA was gestopt en werd overbrugd met heparine. Het bloedingsrisico was even hoog als bij patiënten waarbij de VKA was gestopt en er niet was overbrugd met heparine. Bij hoog-risico tandheelkundige ingrepen was het

bloedingsrisico ruim 3 maal zo hoog als de VKA gecontinueerd werd ten opzichte van wanneer er gestopt werd met de VKA voor de ingreep. Multivariaat analyse toonde aan dat de meeste risicofactoren voor een nabloeding na een tandheelkundige ingreep specifiek gerelateerd waren aan het gekozen perioperatieve beleid. Het risico op trombo-embolische complicaties was laag na tandheelkundige ingrepen, ongeacht het risico van de procedure of de gekozen perioperatieve behandelstrategie.

Hoofdstuk 9,10, 11 en 12 gaan over de anticoagulante effecten van statines en het mogelijk gebruik van deze middelen als alternatieve en veiligere behandeloptie voor veneuze trombose.

In **hoofdstuk 9** wordt het huidige bewijs voor preventie van veneuze trombose middels statines uiteengezet vanuit een epidemiologisch perspectief, waarbij er specifiek wordt ingegaan op potentiële vormen van bias in eerdere klinische studies.

Vervolgens, in **hoofdstuk 10**, hebben we een retrospectieve cohortstudie gedaan naar het effect van statinetherapie op de VKA-onderhoudsdosering in 435 patiënten op fenprocoumon en 303 patiënten op acenocoumarol. De onderhoudsdosering VKA vóór de start met statine behandeling werd vergeleken met de onderhoudsdosering direct na start, na 6 weken en na 12 weken statinetherapie. In fenprocoumon gebruikers was de gemiddelde onderhoudsdosering 0.02 (95% BI, 0.00 to 0.03), 0.03 (95% BI, 0.01 to 0.05) en 0.07 mg/dag (95% BI, 0.04 to 0.09) lager in vergelijking met de onderhoudsdosis vóór aanvang van de statinetherapie. In acenocoumarol gebruikers waren deze doseringen respectievelijk 0.04 (95% BI, 0.01 to 0.07), 0.10 (95% BI, 0.03 to 0.16) en 0.11 mg/dag (95% BI, 0.04 to 0.18) lager. Behandeling met statines was derhalve geassocieerd met minimale, maar statistisch significante veranderingen in VKA-onderhoudsdoseringen, onafhankelijk van het gebruikte type VKA. Hoewel deze dosisveranderingen op zichzelf klinisch niet relevant zijn, zouden deze mogelijk wel verklaard kunnen worden door een anticoagulant effect van statines.

In **hoofdstukken 11 en 12** worden de bevindingen van een gerandomiseerde studie naar de korte termijneffecten van behandeling met rosuvastatine op bloedplaatjes reactiviteit en verschillende stollingsparameters in patiënten die een recente diep veneuze trombose of longembolie hebben doorgemaakt uiteengezet. In de ‘Statins Reduce Thrombophilia’ (START) studie werden patiënten geïncludeerd met een geobjectiveerde veneuze trombose die na 3-12 maanden hun behandeling met VKA mochten staken.

Allereerst, in **hoofdstuk 11**, hebben wij de plaatjesreactiviteit voor en na behandeling met rosuvastatine vergeleken in 50 opeenvolgende patiënten die geïncludeerd waren in de START-trial. Arachidonzuur-gemedieerde plaatjesreactiviteit (PRU) werd gemeten met het VerifyNow system. In totaal hadden 47 van de 50 (94.0%) patiënten een valide PRU-meting bij inclusie en aan het einde van de studie. We vonden geen significant verschil in PRU na afloop van de studie in rosuvastatine gebruikers, noch in de controlegroep. Bovendien was er geen verschil in PRU-verandering tussen de rosuvastatine gebruikers en de controlegroep. Exclusie van deelnemers die antiplaatjestherapie gebruikten of die trombopeen waren, veranderde deze conclusie niet. Derhalve concludeerden wij dat rosuvastatine gebruik geen effect heeft op arachidonzuur-gemedieerde plaatjesreactiviteit.

In **hoofdstuk 12** hebben we het effect van rosuvastatine behandeling op verschillende stollingsparameters onderzocht die samenhangen met leverfunctie (stollingsfactor VII en XI), fibrinolyse (D-dimeer) en endotheelfuntie (Factor VIII en Von Willebrand factor). In totaal werden voor deze studie 255 patiënten geïncludeerd tussen december 2012 en december 2016, van wie er 131 werden behandeld met rosuvastatine en 124 waar geen interventie in plaats vond. Acht van deze deelnemers (5 rosuvastatine gebruikers en 3 controles) voltooiden de studie niet. Derhalve werden in totaal 126 rosuvastatine gebruikers en 121 controles geïncludeerd voor de definitieve analyses. Op baseline waren er geen verschillen tussen de groepen qua onderzochte stollingsparameters. Aan het eind van de studie was het gemiddelde Factor VIII gehalte significant gedaald in de rosuvastatine gebruikers (gemiddeld verschil -7.2 IU/dL, 95% BI -11.5 to -2.9), maar niet in de controlegroep (gemiddeld verschil -0.1; 95% BI -3.0 to 2.9). Correctie voor leeftijd en geslacht veranderden deze resultaten niet. Analoog hieraan was ook het gemiddelde Factor XI gehalte lager geworden in de rosuvastatine gebruikers (-5.9 IU/dL, (95% BI -9.0 to -2.7). Log-getransformeerde D-dimeer waarden waren significant gestegen in de controlegroep (gemiddeld verschil 0.15, 95% BI 0.02 to 0.29), maar onveranderd in de rosuvastatine gebruikers (gemiddeld verschil 0.01, 95% BI -0.08 to 0.10). Er werden geen significante veranderingen in Factor VII of Von Willebrand factor gezien in de intention-to-treat analyses. Restrictie-analyse daarentegen, waar patiënten die een infectie rapporteerden tijdens de studie geëxcludeerd werden, toonde een significante daling van het Von Willebrand factor gehalte in rosuvastatine gebruikers (gemiddeld verschil -7.0; 95% BI -12.7 to -3.2), maar niet in de controlegroep (gemiddeld verschil -1.0; 95% BI -4.3 to 2.4). Vooraf gedefinieerde subgroep analyses toonden allen vergelijkbare resultaten. De anticoagulante effecten waren echter het meest uitgesproken in patiënten met een diep veneuze trombose van het been, patiënten met een niet uitgelokte trombose en in patiënten met cardiovasculaire risicofactoren

ten opzichte van patiënten met respectievelijk een longembolie, een uitgelokte trombose of zonder cardiovasculaire risicofactoren. Concluderend was het gebruik van rosuvastatine geassocieerd met een reductie van verschillende stollingsfactoren in het bloed die geassocieerd zijn met een verhoogd risico op veneuze trombose. Deze anticoagulante effecten bieden een verklaring voor een statine-gemedieerd verlaagd risico op veneuze trombose. Een adequaat gepowerd en gerandomiseerd onderzoek zou verricht moeten worden om definitief uitsluitsel te geven of statinegebruik het risico op een recidief veneuze trombose kan verlagen.

Tenslotte hebben wij in **hoofdstuk 13** de resultaten van de onderzoeken in dit proefschrift bediscussieerd en doen wij aanbevelingen voor verdere onderzoeken om de behandeling met antistolling in de toekomst verder te verbeteren.

APPENDICES

List of publications

Dankwoord

Curriculum Vitae

PhD portfolio

LIST OF PUBLICATIONS

van der Hulle T, Cheung WY, Kooij S, Beenen LFM, van Bemmel T, van Es J, Faber LM, Hazelaar GM, Heringhaus C, Hofstee H, Hovens MMC, Kaasjager KAH, van Klink RCJ, Kruip MJHA, Loeffen RF, Mairuhu ATA, Middeldorp S, Nijkeuter M, van der Pol LM, Schol-Gelok S, Ten Wolde M, Klok FA, Huisman MV; **YEARS study group**. Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study. Lancet. 2017;390(10091):289-297.

van Rein N, **Biedermann JS**, van der Meer FJM, Cannegieter SC, Wiersma N, Vermaas HW, Reitsma PH, Kruip MJHA, Lijfering WM. Major bleeding risks of different low-molecular-weight heparin agents: a cohort study in 12 934 patients treated for acute venous thrombosis. J Thromb Haemost. 2017;15(7):1386-1391.

Biedermann JS*, Rademacher WMH*, Hazendonk HCAM, van Diermen DE, Leebeek FWG, Rozema FR, Kruip MJHA. Predictors of oral cavity bleeding and clinical outcome after dental procedures in patients on vitamin K antagonists. A cohort study. Thromb Haemost. 2017;117(7):1432-1439.
* Contributed equally as first author

Biedermann JS, van den Besselaar AM, van der Meer FJ, Adriaansen HJ, Leebeek FW, Kruip MJ. Control of anticoagulation with VKAs: overestimation of median TTR when assessed by linear interpolation: Reply. Thromb Haemost. 2017;117(4):820-821.

Biedermann JS, van den Besselaar AM, de Maat MP, Leebeek FW, Kruip MJ. Monitoring of treatment with vitamin K antagonists: recombinant thromboplastins are more sensitive to factor VII than tissue-extract thromboplastins. J Thromb Haemost. 2017;15(3):500-506.

Lijfering WM, **Biedermann JS**, Kruip MJ, Leebeek FW, Rosendaal FR, Cannegieter SC. Can we prevent venous thrombosis with statins: an epidemiologic review into mechanism and clinical utility. Expert Rev Hematol. 2016;9(11):1023-1030.

van Rein N, **Biedermann JS**, Bonafacio SM, Kruip MJ, van der Meer FJ, Lijfering WM. Statin use decreases coagulation in users of vitamin K antagonists. Eur J Clin Pharmacol. 2016;72(12):1441-1447.

Biedermann JS, van den Besselaar AM, Leebeek FW, Kruip MJ. Impact of point-of-care international normalized ratio monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: a cohort study: reply. J Thromb Haemost. 2016;14(11):2312-2314.

van den Besselaar AM*, **Biedermann JS***, van der Meer FJ, Adriaansen HJ, Leebeek FW, Kruip MJ. Control of anticoagulation with vitamin K antagonists: overestimation of median time in therapeutic range when assessed by linear interpolation. Thromb Haemost. 2016;116(4):679-686.
* Contributed equally as first author

Biedermann JS, Cannegieter SC, Roest M, van der Meer FJ, Reitsma PH, Kruip MJ, Lijfering WM. Platelet reactivity in patients with venous thrombosis who use rosuvastatin: a randomized controlled clinical trial. J Thromb Haemost. 2016;14(7):1404-1409.

Biedermann JS, van Rein N, van den Besselaar AM, Buhre PN, de Maat MP, van der Meer FJ, Leebeek FW, Kruip MJ. Impact of point-of-care international normalized ratio monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: a cohort study. J Thromb Haemost. 2016;14(4):695-703.

ten Cate-Hoek AJ, Weitz JI, Gailani D, Meijer K, Philippou H, Bouman AC, Whitney Cheung Y, van Mens TE, Govers-Riemslag JW, Vries M, Bleker S, **Biedermann JS**, Stoof SC, Buller HR. Theme 3: Non-invasive management of (recurrent) venous thromboembolism (VTE) and post thrombotic syndrome (PTS). Thromb Res. 2015;136 Suppl 1:S13-8.

van den Besselaar AM, **Biedermann JS**, Kruip MJ. Point-of-care testing and INR within-subject variation in patients receiving a constant dose of vitamin K antagonist. Thromb Haemost. 2015;114(6):1260-1267.

Biedermann JS, Leebeek FW, Buhre PN, de Lathouder S, Barends JP, de Maat MP, van der Meer FJ, Kruip MJ. Agreement between Coaguchek XS and STA-R Evolution (Hepato Quick) INR results depends on the level of INR. Thromb Res. 2015;136(3):652-657.

DANKWOORD

Dit proefschrift is tot stand gekomen met de hulp van vele collega's en inmiddels vrienden. Graag wil ik van de gelegenheid gebruik maken om iedereen te bedanken die dit mogelijk heeft gemaakt.

Allereerst mijn promotor, beste prof. dr. Leebeek, **beste Frank**, als vreemde Amsterdammer kwam ik bij jou terecht voor een nieuw onderzoeksproject. Jouw inzet voor de wetenschap en de dagelijkse patiëntenzorg bewonder ik. Ik waardeer de manier waarop jij mensen verbindt en voor een fijne sfeer in onze onderzoeksgroep zorgt.

Mijn copromotor, beste dr. Kruip, **beste Marieke**. Wat ben ik blij dat jij mij de kans hebt gegeven dit mooie onderzoeksproject samen met jou te mogen uitvoeren. Bijna 4 jaar lang hebben wij ontzettend fijn samen gewerkt om alle onderzoeken uit dit proefschrift te publiceren. Jouw scherpe analytische blik en duidelijke feedback heb ik altijd heel fijn gevonden in onze discussies! Ook als ik het even niet meer zag zitten, zag jij altijd helder perspectief.

Speciale dank aan **prof. dr. H. ten Cate**, **prof dr. S.C. Cannegieter** en **prof dr. K. Meijer** voor het plaatsnemen in de kleine commissie.

Beste dr. van den Besselaar, **beste Ton**. Heel veel dank dat jij afgelopen jaren al jouw expertise met mij hebt willen delen! Onze experimenten in het lab en de fijne overleggen onder een goed bakje koffie zullen mij altijd bij blijven. Vooral hoop ik dat jij vanaf nu heerlijk zult genieten van jouw welverdiende pensioen!

Ook de collega's van de afdeling klinische epidemiologie van het LUMC en de trombosedienst Leiden wil ik graag bedanken. **Willem Lijfering, Nienke van Rein, Prof. Cannegieter, Prof. Rosendaal en dr. van der Meer** veel dank voor de fijne samenwerking de afgelopen jaren!

Grote dank gaat ook uit naar alle collega's bij de trombosedienst van **STAR-SHL** waarmee ik afgelopen jaren heb mogen samenwerken. **Heidi, Alja, Jan-Paul, Mies** en alle andere doseer artsen en adviseurs, dank voor jullie steun, inzichten en alle hulp afgelopen jaren.

Ik wil ook de collega's van het Academisch Centrum Tandheelkunde Amsterdam (ACTA) bedanken voor de samenwerking. Beste **prof. F. Rozema, dr. D. van Diermen en**

Willem Rademacher dank voor het delen van jullie expertise van tandheelkundige ingrepen.

Uiteraard wil ik ook al mijn collega's in het Erasmus MC bedanken. Beste dr. de Maat, beste **Moniek**, dank voor alle keren dat ik met jou heb mogen sparren over mijn lab resultaten. **Dick, Shirley, Simone, Shiraaz en alle collega's van het hemostaselab** dank voor de fijne samenwerking afgelopen jaren op de afdeling. Beste **Albert**, wat hebben we geknokt voor die data van ons predictiemodel. Onwijs knap hoe jij jouw opleiding tot apotheker met promoveren combineert. We doen snel weer een bakje koffie in het Reinier!

Graag wil ik ook nog enkele woorden wijden aan mijn directe collega's en (ex-) kamergenoten: Lieve **Carina**, ik weet nog goed hoe fijn jij mij opving op mijn 1e werkdag. Dank voor alle gezelligheid, steun en fijne gesprekken en discussies die wij gehad hebben samen afgelopen jaren. Geniet met Maarten van jullie mooie nieuwe huis in Barendrecht! Lieve **Carolien**, ik bewonder enorm hoe jij altijd voor iedereen klaar staat met een lach, zelfs als je het zelf stiekem veel te druk hebt. Ook konden wij altijd heerlijk sparren samen over statistiek. Heel veel succes met het veiligstellen van jouw plekje bij de kindergeneeskunde! Lieve **Michelle**, heerlijk hoe jij altijd vrolijk bent, wat er ook gebeurt. Ik hoop jou binnenkort weer eens te zien bij de internisten dagen! Lieve **Janske** en **Yvonne**, hoewel ik maar relatief kort met jullie heb mogen samenwerken, stonden jullie altijd klaar om mij te helpen de eerste maanden, dank! Lieve **Iris, Johan en Caroline**, mijn trouwe koffiekameraden, wat heb ik met jullie gelachen afgelopen jaren om al jullie (en bij vlagen mijn eigen) slechte grappen. Hoe serieus of hard we ook werkten er was altijd tijd voor een welverdiende koffiepauze of een muzikaal of cabaret intermezzo. Ik hoop jullie snel weer te zien! Lieve **Lisette**, hoewel jij liever thee dronk (onbegrijpelijk maar waar) maakte jij onze gezellige kamer natuurlijk compleet. Heel veel succes bij het afronden van jouw mooie project.

Lieve **Mama en Brent**, geweldig was jullie steun en toewijding afgelopen jaren. Mama, mijn discipline en kracht om door te zetten heb ik zeker van jou meegekregen. Brent, dank voor alle goede adviezen en steun op de moeilijke momenten, ik heb er veel van geleerd. **Papa**, hoewel jij helaas dit alles nooit hebt mogen meemaken, hoop ik dat ik ook jou trots heb kunnen maken met deze prestatie.

Lieve **Dominique**, mijn steun en toeverlaat. Al die jaren ben jij, ondanks mijn regelmatige gemopper, er voor mij geweest. Nu wonen wij heerlijk samen in Delft en blijven we bouwen aan onze toekomst samen. Dank dat jij er altijd voor mij was en bent!

Als laatste wil ik graag mijn paranimfen bedanken. Lieve **Eveline en Joost** bedankt voor al jullie hulp met de voorbereidingen op mijn promotie. Joost, al 15 jaar zijn we trouwe vrienden. Van avonden uit tot aan de ontbijttafel bij jouw grootouders om een potje te kaarten. Ondanks dat we elkaar niet meer dagelijks zien, weet ik dat ik altijd bij jou terecht kan. Dank voor jouw vriendschap!
Eveline, lieve zus, wat was jij enthousiast om paranimf te mogen worden! Een goede psychiater aan je zijde op zo een spannende dag is natuurlijk nooit weg. Lang heb ik jouw goede voorbeeld gevolgd, maar nu heb ik toch echt mijn eigen route gekozen. Dank dat jij er altijd voor me bent!

CURRICULUM VITAE

Joseph Siegmund Biedermann werd op 25 april 1989 geboren te Amstelveen. Na het behalen van het gymnasium diploma aan het Vossius Gymnasium te Amsterdam, studeerde hij vanaf 2007 geneeskunde aan de Vrije Universiteit in Amsterdam. In 2013 heeft hij 6 maanden wetenschappelijk onderzoek verricht bij de afdeling endocrinologie van het VU-medisch Centrum onder begeleiding van dr. E.M. Eekhoff, naar de effecten van lichamelijke conditie op overleving en cardiovasculaire mortaliteit in ouderen. Na zijn afstuderen begon hij in November 2013 aan zijn promotie onderzoek onder supervisie van Dr. M.J.H.A. Kruip en Prof. Dr. F.W.G Leebeek. Dit promotie onderzoek heeft geleid tot het proefschrift dat voor u ligt. Sinds 1 april 2017 is hij werkzaam als arts-assistent Interne Geneeskunde in het Reinier de Graaf Gasthuis te Delft.

PHD PORTFOLIO

PHD TRAINING

	Year	Workload (ECTS)
General academic skills		
Biomedical English Writing Course	2015	3,0
Course Research Integrity	2013	0,3
Research skills		
Basic course on Regulations and Organization for Clinical Investigators (BROK)	2015	1,5
Open Clinica training	2015	0,3
In-depth courses (e.g. Research school, Medical Training)		
COEUR course: Cardiovascular Medicine	2013	1,5
COEUR course: Pathophysiology of ischemic heart disease	2014	1,5
COEUR course: Cardiovascular Clinical Epidemiology	2014	1,5
3x NVTH courses on Thrombosis and Hemostasis	2014-2016	3,0
Presentations		
Dutch Hematology Congress	2015	0,8
ECTH	2016	0,8
NVTH	2015	0,8
ECAT symposium (invited speaker)	2016	1,2
Impact of Point-of-Care INR monitoring, Roche Diagnostics, Almere (invited speaker)	2015	0,8
2x FNT meeting on quality control and assurance (invited speaker)	2014, 2016	0,8
Nurses symposium on anticoagulation 2x (invited speaker)	2014, 2016	1,6
4x poster presentations (2x ISTH 2015, 1x SSC 2016, 1x ECTH 2016)	2015	1,2

Conferences and symposia		
ISTH	2015	1,8
2x NVTH	2014, 2015	1,2
Dutch Hematology Congress	2015	0,3
2x Federation of Dutch Thrombosis Services	2014,2016	0,6
ECTH	2016	0,9
2x AMSTOL symposium	2014, 2016	0,6
COEUR PhD day	2014	0,5
Clinical Chemistry Symposium	2016	0,3
Maastricht consensus conference on thrombosis	2015	0,3
Seminars and workshops		
2x COEUR research seminar	2013, 2014	1,0
5x COEUR lecture	2013-2016	1,5
16th regional post-graduate course for hematologists, Rotterdam	2014	0,3
NVTH PhD day	2014	0,4
Hematology PhD training		
Work discussions and literature discussions (weekly)	2013-2017	5,0

TEACHING ACTIVITIES

Lecturing		
2x Education for thrombosis service physicians (FNT) (invited speaker)	2014, 2016	1,6
10x Coagulation lecture for nurses (EMC)	2013-2017	1,0
Supervision of medical students		
Review 2nd year medical students during elective course on blood coagulation for 2nd year medical students (EMC)	2016, 2017	0,5
Other		
Scientific editing and meetings for FNT	2015-2017	2,0
Total		40,5